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Acta Pædiat Scand 60 1–5 1971

THE DECREASE IN THE SERUM BILIRUBIN LEVEL IN PREMATURE INFANTS BY OROTIC ACID

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The prevention of neonatal hyperbilirubinaemia has been the subject of intensive recent work in*volving a search for inductors of the hepatic* glucuronyl transferase system which might reasonably be used in a clinical setting The use of phenobarbital (2 18 19 20) in premature infants with disorders of respiration is not indicated because of the depressive effect of the barbiturate on respiration The antenatal administration of phenobarbital to the mother the effect of which on the newborn is also proved (11) meets with considerable difficulties in delivery before term Edema develops with the use of phenylbutazone (4 12) and it likely causes a displacement of the bilirubin out of its protein binding in the serum therefore its clinical administration cannot be advised It is true that nikethamide in a therapeutic dose is free from side effects however its effect is too small to achieve an essential clinical advantage (6 9 16) Thus we have looked into other agents which might prevent an increase of the serum bilirubin into toxic ranges Besides the immaturity of the glucuronyl transferase system the deficiency of the uridine-coenzyme is also important in the genesis of the hyperbilirubinemia The synthesis of the UTP passes from asparaginic acid through the phase of orotic acid Thus Brodersen recommended examination of the effect of orotic acid in newborn infants (1) This substance might induce the formation of glucuronyl transferase because orotic acid is the precursor of the pyrimidine bases cytosine thymine and urazile which are necessary for the synthesis of the nucleic acids and it was pointed out that the rate of synthesis of the RNA increases after the application of orotic acid (14) In addition to this substitution effect there may also be an inductive effect of the enzyme Up to now only Japanese authors (11) have investigated the influence of orotic acid on the bilirubin level of mature new borns They found a small and partially significant decrease in the bilirubin level after treatment with a daily dose of 200 mg of orotic acid in a very small number of new borns

In our first investigations we have therefore examined the influence of a medication with orotic acid We administered a daily dose of 100 mg in premature infants from the 1st–6th day of life The reaction of the coagulation factors was not influenced by this dosage therefore we got no indication of an enzyme inductive effect An influence on the serum bilirubin level was however detectable 15 exchange transfusions were required in the control group (54 premature infants) whereas this was necessary in only 6 cases in the other group (56 premature infants) that has been treated with orotic acid (5) An increase in the dose of orotic acid seemed justifiable because we did not detect any side effects and because of the experiences that were gained in the animal ex

Table 1 *Distribution of birth weights*

Birth weight	1 200–1 500 g	1 500–2 000 g	2 000–2 500 g	Total
Orotic acid group	8	54	40	102
Control group	12	54	36	102

periment (3, 10 17, 21) respectively, in the administration of orotic acid to adults (13) Therefore we increased the daily dosage of orotic acid up to 300 mg in our further investigations that are here discussed

METHOD

Premature infants with a birth weight of 1 200-2 500 g were treated in the same manner as in the preceding investigations One group of children was treated with a daily dose of 300 mg of orotic acid¹ from the first till the sixth day orally given in two single doses The other group of children was not treated Half of the children were chosen in an alternating manner and afterwards by the formation of groups whereby no selection was made Feeding method and intensive care of the premature infants were practised identically in both groups The total bilirubin as well as the indirectly reacting bilirubin in the serum was analysed according to the method of Jendrassik & Grof and Cleghorn respectively in all premature infants from the third till the sixth day of life A blood exchange transfusion was performed at an increase in the indirectly reacting bilirubin of over 18 mg In 10 premature infants of the control group and in 15 premature infants who had been treated with orotic acid a determination of the activity of the liver depending coagulation factors II and VII and of the factor V was performed (utilizing Behring werke reagents) in each case on the fifth day

RESULTS

A sum total of 223 premature infants was covered by the investigations Out of 111 premature infants of the orotic acid group 9 children died from the first to the third day of life, so that 102 premature infants were at our disposal for the evaluation of the serum bilirubin levels and the calculation of the blood exchange frequency Out of 112 premature infants of the control group 10 children died within the first 3 days of life so that 102 premature infants without a treatment with orotic acid could be compared with the other group The distribution of the birth weights in both groups showed no significant differences (Table 1)

Frequency of the blood exchange transfusion

An exchange transfusion had to be performed in only 4 children out of 102 premature infants of the orotic acid group, whilst this was necessary in 30 cases out of 102 premature infants of the control group The difference in the exchange frequency of both groups is statistically most significant on an assumption of a probability of error of $p = 0.1\%$ ($\chi^2 = 23.8$)

Reaction of the serum bilirubin

Arithmetical average value and standard deviation of the total and of the indirectly reacting

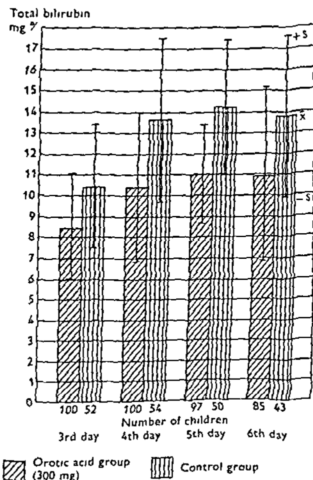


Fig 1 *Total bilirubin values in orotic acid treated group and in control group*

¹ A test preparation of the VEB Jenapharm

bilirubin were separately evaluated in the 3 weight groups for the 3rd–6th day of life The bilirubin values performed after blood exchange transfusions were not taken into consideration in these calculations Some high bilirubin levels of the control group must be omitted from the calculation for this group because blood exchange transfusions must be performed significantly more frequently in the premature infants of this group There were no significant differences of the bilirubin levels between the 3 formed weight groups as well within the orotic acid group as within the control group Therefore a total comparison of the average values of the orotic acid group and of the control group was possible As it appears from the diagrams (Figs 1 and 2) the average values of the total and of the indirectly reacting bilirubin are distinctly lower in the orotic acid group from the 3rd–6th day of life This difference is statistically significant for the 3rd–6th day on an assumption of a probability of error of $p=1\%$ (t test)

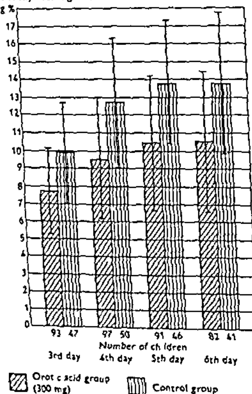


Fig 2 Indirectly reacting bilirubin values in orotic acid treated group and in control group

Table 2 *Reaction of coagulation factors*

	F II	F VII	F V
Orotic acid group 300 mg/daily			
$n=15$			
x	38	37	8?
s	8 9	7 9	17 4
Control group without orotic acid			
$n=10$			
x	41	34	78
s	13 2	16 6	28 3

Reaction of the coagulation factors

The activity of the liver-depending coagulation factors II and VII which was determined on the 5th day of life as well as the activity of factor V were equal in both groups (Table 2)

DISCUSSION

The results of our investigations show that action of orotic acid on serum bilirubin values could be essentially intensified by increasing the daily administered doses of orotic acid *from* 100 to 300 mg The total and indirect bilirubin values on the 3rd–6th day of life are statistically significant lower than in the control group Hereby must be considered that some high bilirubin values in the control group fall outside and cannot be brought into calculation because of performed exchange transfusions

In respect to the essential blood exchange transfusions a very considerable decrease in the frequency could be achieved in the children who had been treated with orotic acid In this group only 4 exchange transfusions were necessary whereas 30 exchange transfusions were required in the control group The advantages of such a reduction are evident

Before however an application of orotic acid can be generally advised the question of the side effects must be answered As mentioned above orotic acid is a compound that the body

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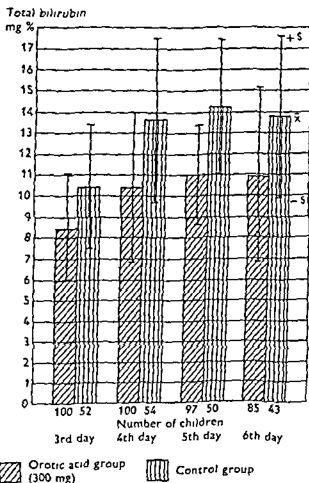


Fig. 1. Total bilirubin values *in orotic acid treated group and in control group.*

[1] A test preparation of the VEB Jenapharm.

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itself synthesises in the synthesis of the nucleic acids. As the name suggests (oros = whey) orotic acid can also be found in cow's milk at an average of 50–100 mg per liter. A daily dose up to 3–5 g of orotic acid was administered to patients, who suffered from gout (13), without the observation of side effects. In the animal experiment, however, fatty degenerations of the liver (3, 10, 17, 21) and crystallisation of the orotic acid in the renal tubuli were detected after the administration of very high doses of orotic acid (15).

The clinical observation of the premature infants who had been treated with orotic acid in our hospital showed no detrimental effects. In the orotic acid group the mortality of 16 children from the 1st–10th day of life was practically on the same level with the mortality in the control group in which 15 children died. Pathologic anatomical control examinations were performed in the 7 premature infants of the orotic acid group who died within the 4th–10th day of life. No alterations were observed that could be charged to the use of orotic acid. We think, however, it is necessary to examine the question of side effects still more carefully before orotic acid is routinely administered to prevent premature infants from contracting hyperbilirubinemia. We ourselves have already started such studies which will also assist in deciding whether a renewed increase in the daily dose of orotic acid can be justified. It has been proved by our investigations that the effect of this substance depends upon the dose administered. The mode of action of the orotic acid is dubious up till now. Our investigations showed that the activity of the liver depending coagulation factors II and VII could not be increased by a dose of 300 mg compared with a control group. The coagulation factors and glucuronyl transferase are formed in the liver cell on the same spot, namely in the endoplasmatic reticulum. It has been proved that on application of the enzyme inductor phenobarbital there was an increase in the glucuronyl transferase activity simultaneously with an increase in the activity of the coagulation factors that are formed in the liver (12). The absence of an increase in the activity of the coagulation factors that has been demonstrated by us, makes an inductive effect of the orotic acid not very likely. The results of the investigations of Klinger who did not find an enzyme inductive effect of the orotic acid in rats (8) correspond with our investigations. In our opinion the effect of the orotic acid is primarily a substitution effect on the coenzyme of the glucuronyl transferase, the UTP. With the co-operation of other clinics and institutes we are attempting to further elucidate this question.

SUMMARY

The influence of higher doses of orotic acid on the serum bilirubin level of premature infants was investigated following studies with a lower dose. 102 premature infants were treated with a daily dose of 300 mg of orotic acid from the 1st–6th day after birth. An equal number of children served as a control group. The serum level for the indirect bilirubin that was analysed from the 3rd–6th day of life could be statistically significantly decreased by the administration of orotic acid. Blood exchange transfusions were necessary only four times with the administration of orotic acid, whereas blood exchange transfusions were required in 30 premature infants of the control group. The question of eventual side effects and the supposed mode of action of the orotic acid are discussed.

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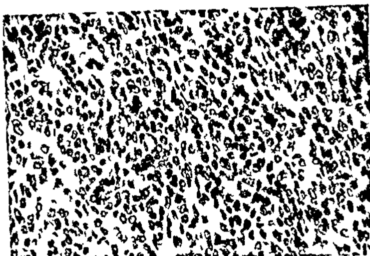


Fig 1 The tumor is made of plump fusiform cells with a tendency to a fasciculated or whorly arrangement Case 1 Hematoxylin phloxin saffron ×150

cells with large rounded or elongated nuclei (Fig 1) There was no evidence of anaplasia or abnormal mitoses In some areas the tumor was composed of dense cellular agglomerations lobulated by collagen septa Elsewhere the cells were more widely separated by edema or hemorrhagic infarction and there were macrophages containing hemosiderin in the peripheral areas None of the sections disclosed a rosette or folliculoid pattern suggestive of a granulosa cell component Foot s stain revealed that individual tumor cells were surrounded by reticulin furnishing further confirmation of this impression (Fig 2) Intra and extra cellular lipoid material was demonstrated by Sudan III on frozen sections (Fig 3) Normal remnants of ovarian tissue were not discernible Sections of the uterus and oviducts were unavailable Diagnosis bilateral thecoma

Case 2

Stella D was referred to Children s Hospital at 3½ years of age for generalized seizures The attacks were refractory to phenobarbital (40 mg daily) and as a result the child was placed on a combination of diphenylhydantoin and phenacetylurea The convulsions were controlled however petit mal attacks supervened Treatment was changed to phenobarbital (100 mg daily) and phenacetylurea (0 9 g daily) Toxic hypertrophy of the gums developed but treatment was not interrupted

Four months later in February 1965 the child s mother noted a rapid increase of her daughter s weight This was accompanied by facial edema and subsequent abdominal distension for which the child was readmitted to the hospital

Physical examination revealed a well developed 6½ years old female (117 cm 21 kg) with no evidence of precocious puberty The abdomen was grossly distended and paracentesis produced 1 500 ml of bloody fluid which contained lymphocytes and endothelial cells the protein concentration was 3 6 g/100 ml After this procedure a large suprapubic mass was palpable also found on rectal examination The I V P and



Fig 2 Individual cells are surrounded by reticulin fibers which do not demonstrate any follicular pattern Case 1 Foot s stain 600

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BILATERAL NON FUNCTIONNING THECOMA OF THE OVARY IN EPILEPTIC CHILDREN UNDER ANTICONVULSANT THERAPY

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JEAN LEMERLE JEAN M WATCHI and PHILIPPE SERINGE

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In a personal series of malignant ovarian tumors occurring in childhood we have observed two most unusual cases These involved young girls who presented with bloody ascites asso ciated with bilateral ovarian tumors In each case surgical excision was curative and histo logical examination of the tumors revealed pure ovarian thecomas Both children were epileptic and had been on long term anticonvulsant therapy

The similarity of the clinical and pathologi cal features of the 2 cases was so striking that it suggested a possible non fortuitous associa-tion between anti convulsant therapy and the development of ovarian thecoma This possi bility was given further credence by our dis covery of an almost identical case in the litera ture (1)

The purpose of this article is to acquaint pediatricians with our observations if indeed there is an association between apilepsy or *which is more probably anti convulsant therapy* and the development of ovarian thecoma in childhood it may be anticipated that similar cases will be reported

CASE REPORTS

Case 1

Maud B (I G R 12/60 2465) a 5½ year old female had generalized seizures at 9 months of age and again at 19 months of age Initial treatment with phenobarbital 40 mg daily was given However at 3 years of age she developed petit mal seizures for which she was started on trimethadione 0 6 g daily Phenacetylurea, 0 9 g daily was subsequently added for better control of the petit mal The child had been on this regimen for 6 months when she was referred to our hospital in April 1960 because of ascites This had been detected 3 days prior to admission in connection with an episode of transient facial edema

Physical examination revealed a normally developed 5½ year old girl (108 cm 19 5 kg) with no evidence of abnormal sexual development The abdomen was grossly distended the left iliac fossa being somewhat firmer to palpation than the right The remainder of the physical examination was unremarkable Two abdominal paracenteses prior to admission had effected the removal of a total of 1 300 ml of bloody fluid A third paracentesis of 1 000 ml following admission permitted palpation of a large tumor in the left iliac fossa The fluid contained numerous red cells macrophages and endothelial cells and a few clusters of basophilic cells suggesting malignancy The protein concentration was 3 2 g/100 ml

The I V P and chest film were normal as was examination of the bone marrow Coagulation studies revealed normal coagulation and bleeding times as well as a normal prothrombin complex Clot retrac tion was absent and clot lysis occurred in 30 min at 37 C The test for fibrinolysis revealed it was almost complete in 2 hours

Laparotomy disclosed bilateral solid ovarian tumors with distinct areas of hemorrhage and infarction The tumors measured 10×12 cm and 6×9 cm on the left and right sides respectively Bilateral oophorec tomy and salpingectomy with subtotal hysterectomy were performed Uterine size was normal and there was no evidence of abdominal metastasis The post operative course was uneventful and the coagulation abnormalities were corrected immediately The child is well 10 years later with good control of the epi lepsy

Microscopic findings of both tumors were similar they were composed of bundles of plump spindle

Table 1 *Anti epileptic drugs given to the three patients*

Drug	Trade name	Case 1	Case 2	Faber s case
Phenobarbital	Phenobarbital	+	+	+
Trimethadione	Tridione	+	0	+
Paramethadione	Paradione	0	0	+
Phenacetylurea	Phenacemide	+	+	0
Phensuximide	Milontin	0	0	+
D heryl hydantoin	Dilantin	0	+	+

she was treated with diphenylhydantoin and phenobarbital before being changed to para dione (2) She was admitted to the hospital in August, 1949 with orbital and pedal edema abdominal ascites develop d subsequently The presence of a malar butterfly rash in addition to hematuria and proteinuria initially suggested drug toxicity Following paracentesis a large supra pubic mass became palpable At laparotomy bilateral tumors were resected with pre servation of the uterus Pathological examina tion of the ovaries was similar to that of our own cases and also consistent with the diag nosis of thecoma The child has been followed for 10 years and has remained well with the exception of refractory petit mal seizures

Ovarian thecoma is a neoplasm which devel ops from the ovarian mesenchyme it is most often associated with morphological evidence of granulosa cell development In cases of 'pure thecoma two d fferent pathological en tities must be ruled out thecal hyperplasia and fibroma of the ovary

The diagnosis of thecal hyperplasia was pro posed by the Armed Forces Institute of Pathology (Washington D C) on the basis of th ir review of the slides of our second case They pointed out that "these ovarian changes would occur in association with torsion of the ovarian adnexal structure the ovary becoming tremen dously enlarged secondary to venous obstruc tion" (8) Adnexal torsion was not observed in any of the three cases and therefore we are reluctant to accept this diagnosis

We also entertained the diagnosis of fibroma of the ovary however several inconsistencies mitigated against this diagnosis as well areas of hemorrhage were more prominent in our cases than would normally be expected in ovarian fibroma a fasciculated pattern of the cells was not evident the tumor cells were plump and cytologically quite distinct from fibroblasts Finally in case 1 as in Faber s case special stains for intra and extra cellular lipids were positive the presence of fat in a mesenchymal ovarian tumor is generally accepted as the best microscopic criterion in support of the diagnosis of thecoma as opposed to fibroma

Although bilateral theca granulosa cell tu mors associated with precocious puberty are well known (10) Faber s case is the only case of bilateral pure thecoma without endocrine activity we have found in the pediatric litera ture

The lack of feminizing activity in these 3 cases of pure ovarian thecoma is difficult to explain The nature of hormonal synthesis in ovarian tumors is not well understood Ryan (6) has postulated that thecal cells require the adjuvant presence of granulosa lutein cells or other sources of progesterone for them to be come functionally active Thorough examina tion of the pathological material of our cases failed to reveal a granulosa-cell component, and this could explain the lack of endocrine symptomatology

The children under discussion were epileptic two suffered from grand mal and all three of them from petit mal epilepsy None of the chil dren revealed neurological evidence of an or ganic brain disorder We are unaware of a genetic relationship between ovarian tumors and epilepsy Miller & Fraumeni (5) in review ing the charts of children with ovarian tumors found only one case in which a teratoma of the ovary was associated with microcephaly mental retardation and epilepsy In our per sonal experience with 40 cases of ovarian tu mors in children only the 2 cases of pure thecoma were associated with epilepsy Never theless we recognize that a relationship be tween some cerebral anomalies and ovarian tumors cannot be entirely ruled out, because

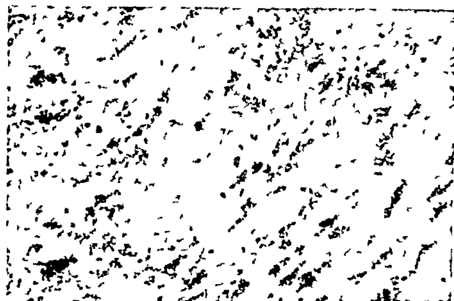


Fig 3 Lipidization is obvious most of the fat droplets are intra cellular Case 1 Sudan III on frozen section × 150

chest X ray were within normal limits as were routine examinations of the blood and urine

At laparotomy it was apparent that the residual ascites was associated with bilateral ruptured ovarian tumors Bilateral oophorectomy and subtotal hysterec tomy were performed the remainder of the abdominal cavity was unremarkable The post operative course was uncomplicated except for incomplete control of the epilepsy and the child is doing well 2 years later A test for lymphoblastic transformation of lympho cytes *in vitro* in the presence of diphenylhydantoin was negative

The pathological specimen consisted of bilateral ovarian tumors weighing 90 and 100 g respectively both revealed gross areas of hemorrhagic infarction (Fig 4) The microscopic appearance was similar to that of the first case and also there was no evidence of a granulosa cell component Specially stained sec tions did not reveal the presence of lipoid material in the available sections Remnants of normal ovarian tissue with normal primary follicles were discernible in one area The uterus was of normal size with an immature endometrium and no evidence of glandular or stromal hyperplasia the oviducts were normal Diagnosis bilateral thecoma

DISCUSSION

A case almost identical to the above two has been reported by Faber (1)

A 3-year old girl developed petit mal epilepsy in December, 1948 She was treated with tri methadione from January to March, 1949 fol lowing which she received a short course of succinimide Between March and August 1949

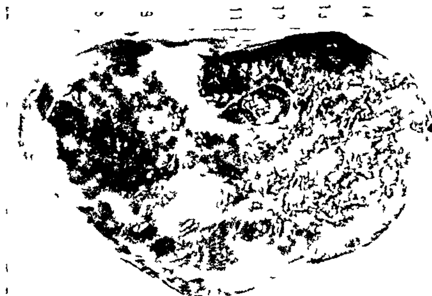


Fig 4 Gross section of the left tumor in case 2 Numerous pseudocystic areas after infarction and hemorrhages

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Phensuximide	Milontin	0	0	+
Diphenyl hydantoin	Dilantin	0	+	+

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The lack of feminizing activity in these 3 cases of pure ovarian thecoma is difficult to explain The nature of hormonal synthesis in ovarian tumors is not well understood Ryan (6) has postulated that thecal cells require the adjuvant presence of granulosa lutein cells or other sources of progesterone for them to be come functionally active Thorough examina tion of the pathological material of our cases failed to reveal a granulosa-cell component, and this could explain the lack of endocrine symptomatology

The children under discussion were epileptic two suffered from grand mal and all three of them from petit mal epilepsy None of the chil dren revealed neurological evidence of an or ganic brain disorder We are unaware of a genetic relationship between ovarian tumors and epilepsy Miller & Fraumeni (5) in review ing the charts of children with ovarian tumors found only one case in which a teratoma of the ovary was associated with microcephaly mental retardation and epilepsy In our personal experience with 40 cases of ovarian tu mors in children, only the 2 cases of pure thecoma were associated with epilepsy Never theless we recognize that a relationship be tween some cerebral anomalies and ovarian tumors cannot be entirely ruled out, because

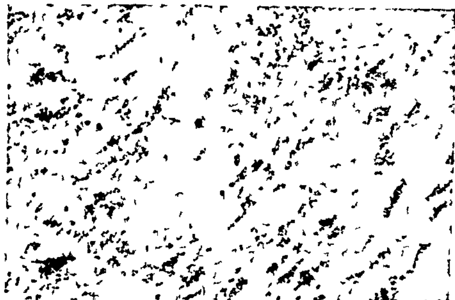


Fig 3 Lipidization is obvious most of the fat droplets are intra cellular Case 1 Sudan III on frozen section × 150

chest X ray were within normal limits as were routine examinations of the blood and urine

At laparotomy it was apparent that the residual ascites was associated with bilateral ruptured ovarian tumors Bilateral oophorectomy and subtotal hysterec tomy were performed the remainder of the abdominal cavity was unremarkable The post operative course was uncomplicated except for incomplete control of the epilepsy and the child is doing well 2 years later A test for lymphoblastic transformation of lympho cytes *in vitro* in the presence of diphenylhydantoin was negative

The pathological specimen consisted of bilateral ovarian tumors weighing 90 and 100 g respectively both revealed gross areas of hemorrhagic infarction (Fig 4) The microscopic appearance was similar to that of the first case and also there was no evidence of a granulosa cell component Specially stained sec tions did not reveal the presence of lipoid material in the available sections Remnants of normal ovarian tissue with normal primary follicles were discernible in one area The uterus was of normal size with an immature endometrium and no evidence of glandular or stromal hyperplasia the oviducts were normal Diagnosis bilateral thecoma

DISCUSSION

A case almost identical to the above two has been reported by Faber (1)

A 3 year old girl developed petit mal epilepsy in December, 1948 She was treated with tri methadione from January to March 1949 fol lowing which she received a short course of succinimide Between March and August 1949

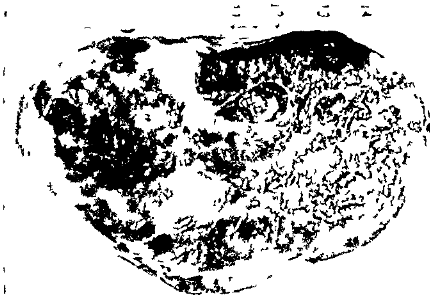


Fig 4 Gross section of the left tumor in case 2 Numerous pseudocystic areas after infarction and hemorrhages

Table 1. *Anti epileptic drugs given to the three patients*

Drug	Trade name	Case 1	Case 2	Faber s case
Phenobarbital	Phenobarbital	+	+	+
Trimethadione	Tridione	+	0	+
Paramethadione	Paradione	0	0	+
Phenacetylurea	Phenacemide	+	+	0
Phensuximide	Milontin	0	0	+
Diphenyl hydantoin	Dilantin	0	+	+

she was treated with diphenylhydantoin and phenobarbital before being changed to paradione (2) She was admitted to the hospital in August, 1949 with orbital and pedal edema abdominal ascites developed subsequently The presence of a malar butterfly rash in addition to hematuria and proteinuria initially suggested drug toxicity Following paracentesis a large supra pubic mass became palpable At laparotomy bilateral tumors were resected with preservation of the uterus Pathological examination of the ovaries was similar to that of our own cases and also consistent with the diagnosis of thecoma The child has been followed for 10 years and has remained well with the exception of refractory petit mal seizures

Ovarian thecoma is a neoplasm which develops from the ovarian mesenchyme it is most often associated with morphological evidence of granulosa cell development In cases of "pure" thecoma two different pathological entities must be ruled out thecal hyperplasia and fibroma of the ovary

The diagnosis of thecal hyperplasia was proposed by the Armed Forces Institute of Pathology (Washington D C) on the basis of their review of the slides of our second case They pointed out that "these ovarian changes would occur in association with torsion of the ovarian adnexal structure the ovary becoming tremendously enlarged secondary to veinous obstruction (8) Adnexal torsion was not observed in any of the three cases and therefore we are reluctant to accept this diagnosis

We also entertained the diagnosis of fibroma of the ovary however several inconsistencies mitigated against this diagnosis as well areas of hemorrhage were more prominent in our cases than would normally be expected in ovarian fibroma a fasciculated pattern of the cells was not evident the tumor cells were plump and cytologically quite distinct from fibroblasts Finally in case 1 as in Faber s case special stains for intra and extra-cellular lipids were positive the presence of fat in a mesenchymal ovarian tumor is generally accepted as the best microscopic criterion in support of the diagnosis of thecoma as opposed to fibroma

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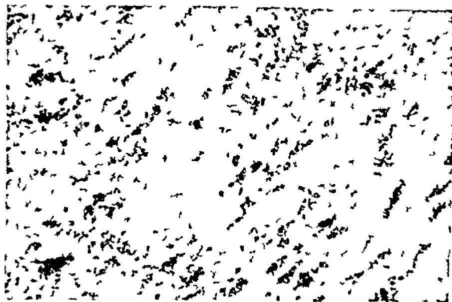


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MILD AND SEVERE PKU COMPARATIVE STUDIES IN TWO INFANTS

O HJALMARSSON R. JAGENBURG and S RÖDJER

From the Departments of Paediatrics and Clinical Chemistry University of Gothenburg Gothenburg Sweden

With the introduction of masscreening surveys of newborns for early detection of phenylketonuria an increasing number of children with hyperphenylalaninemia is detected Not only the incidence of classical phenylketonuria (PKU) has been found to be greater than previously regarded (6) but a considerable number of cases have been found to have a moderate increase of the blood phenylalanine level which has not always led to the serious consequences seen in children with untreated PKU (1 2 3 7 10 12 18 19 20) Many of these cases no doubt differ from classical PKU but there is no agreement about the classification of the different forms of hyperphenylalaninemia WHO recommended in 1968 (24) a classification into four groups

1 classical PKU—here called *classical severe PKU*

2 classical PKU with 2–3 times greater tolerance of phenylalanine than in the previous form—here called *classical mild PKU*

3 *transient hyperphenylalaninemia* with or without phenylpyruvicaciduria and with or without mental retardation and

4 *persistent hyperphenylalaninemia* without phenylpyruvicaciduria or mental retardation

This classification is based on both clinical and or laboratory findings There are no definite limits for the plasma phenylalanine level separating the groups because the level varies considerably with age and the phenylalanine intake

This paper describes a study of two infants with hyperphenylalaninemia detected during their first week of life One of them was a girl with a classical severe PKU She had a very high blood concentration at 5 days of age and diet therapy was immediately started The other infant had slowly increasing plasma phenylalanine values amounting to 16 mg/100 ml at 3 weeks of age There was no phenylpyruvic aciduria The changes in the plasma phenylalanine level during fasting after ordinary meals and after intravenous phenylalanine infusions were determined in order to disclose any differences in the phenylalanine turn over in the two infants The studies were performed when the infants were between 2–5 weeks of age

CASE REPORTS

Case K J This girl is the third child of healthy not consanguineous parents No mental retardation or disease is known in the family The patient s two siblings are normal The infant was born at term birth weight 3 300 g The gestation and the delivery were normal The infant was initially breast fed A Guthrie test for PKU at 5 days of age showed a blood phenylalanine level of more than 20 mg/100 ml increasing to 47 mg/100 ml before treatment was started at the age of 11 days Other amino acids including tyrosine were normal The phenylalanine content of the food had to be considerably reduced to reach a suitable blood level (*Fig 1*) From the third month of life a phenylalanine intake of about

complete neurological and radiological studies were not performed in our reported cases

The role of anti-convulsant therapy in the development of ovarian thecoma is more interesting to consider Table 1 lists the drugs received by our patients These drugs have been reported as able to produce pseudo lymphomas (7) true lymphomas (4), and even pseudo pseudo lymphomas (3) hydantoin derivatives are the most frequently implicated These similar side effects illustrated by the above group may possibly be explained on basis of the common structural features (phenol group) (9) present in most of the anti convulsant drugs

Both in the cases of the lymphomatous and ovarian growths found in patients treated with anti convulsant drugs the question can be discussed whether there is a true or a pseudo tumor

It is certain, however that if ovarian thecomas indeed represent a side effect of anti convulsant therapy they are extremely rare in proportion to the large number of patients on treatment for epilepsy

According to the low malignant potential of granulosa theca cell tumors the outlook for survival in these cases has been good with surgery alone Hysterectomy was unnecessary in our 2 cases and it should be avoided but the surgeons were impressed by the gross appearance of the ovaries and their association with bloody ascites

Bilateral oophorectomy is quite undesirable in a young female and one wonders whether withdrawal of anti convulsant therapy might permit the regression of the ovarian changes as has been described in connection with pseudo lymphomatous adenopathy If one could be relatively certain of the clinical diagnosis this would be a valid trial however the rapid evolution of clinical signs and symptoms as well as the striking changes of the ovaries noted at laparotomy tend a priori to make the regression of the tumors rather dubious In any case we believe that surgery should be as conservative as possible

SUMMARY

The peculiar features of two bilateral thecoma of the ovary are described They were discovered because of a rapid enlargement of the abdomen with hemorrhagic ascites, but without any sign of precocious puberty They occurred in young females treated with anti-convulsant therapy for epilepsy They have been cured with surgery alone

A third case has been found in the literature Discussion is raised about the meaning of this association The possible relationship with side effect of anti epileptic drugs is suggested

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Case A J This girl is the third child of healthy not consanguineous parents No mental retardation or disease is known in the family The patient s two siblings are normal The infant was born at term birth weight 3 300 g The gestation and the delivery were normal The infant was initially breast fed A Guthrie test for PKU at 5 days of age showed a blood phenylalanine level of more than 20 mg/100 ml increasing to 47 mg 100 ml before treatment was started at the age of 11 days Other amino acids including tyrosine were normal The phenylalanine content of the food had to be considerably reduced to reach a suitable blood level (Fig 1) From the third month of life a phenylalanine intake of about

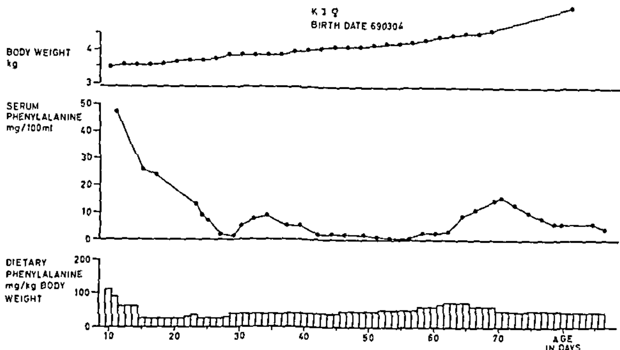


Fig 1 Weight curve blood phenylalanine level and calculated phenylalanine intake in patient K J

40 mg per kg and day gave a blood level of 4-7 mg/100 ml The patient is now 10 months old The weight gain her mental and neurological status and EEG registrations are normal

Case S S This girl is the third child of healthy parents One possibly two of siblings of her maternal grandfather were mentally retarded had pareses and died before 20 years of age The parents are not con sanguineous and the siblings are healthy and nor mally developed The fasting plasma phenylalanine level is normal in the parents and the siblings The infant was born at term birth weight 3 300 g The gestation and the delivery were normal She was ad mitted to hospital 9 days old because a phenylalanine concentration of 10 mg/100 ml had been found by the Guthrie method in a blood sample obtained at the age of 5 days During the first 20 days of life the girl was breast fed ad lib The calculated phenyl alanine intake was between 120 and 160 mg per kg body weight and day (Fig 2) On this diet the blood phenylalanine level slowly increased from 10 to 16 mg/100 ml Apart from the elevated phenylalanine level the plasma amino acids including tyrosine were normal Phenylpyruvic acid o hydroxyphenylacetic acid and phenylacetylglutamine were not found in the urine On account of the increasing level of phe nylalanine in the blood the intake of this amino acid was reduced to 80 mg per kg and day by replacing half the milk volume by Lofenalac® which resulted in an immediate decrease of the phenylalanine level

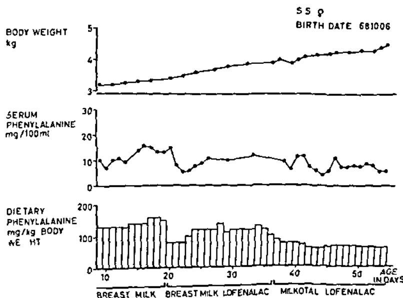


Fig 2 Weight curve blood phenylalanine level and calculated phenylalanine intake in patient S S

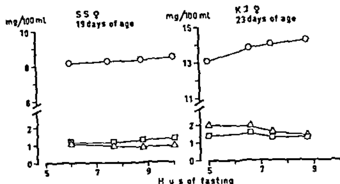


Fig 3 Changes in the plasma levels of phenylalanine (o—o) tyrosine (Δ—Δ) and leucine (□—□) during fasting Zero time corresponds to 2 o clock in the night when the last meal was given

to 6 mg/100 ml The phenylalanine intake was increased to about 120 mg per kg and day during the following 12 days and this gave a serum level of about 10 mg/100 ml To reach a level of 4-7 mg/100 ml the intake was later reduced to about 60 mg phenylalanine per kg and day The weight gain and somatic development during the period of study were normal At the age of 4 weeks a slight muscular hypertonia without definite pathological significance was noticed EEG registrations were normal Eight weeks old the infant was discharged from the hospital and received continued dietary treatment at home During the third month of life the phenylalanine intake had to be gradually reduced to about 40 mg per kg and day to keep the blood level within 4 7 mg per 100 ml

METHODS

Phenylalanine was either measured by the Guthrie method (8) for daily routine (Figs 1 and 2) or by ion exchange chromatography (Figs 3-6) using a Beckman amino acid analyzer model 120 C (21) A short program specially adapted for determination of tyrosine and phenylalanine was run For these determinations blood was obtained by internal jugular vein puncture Heparin was used as an anticoagulant The plasma proteins were precipitated by picric acid (22) The urine was analyzed for the presence of phenylpyruvic acid by Phenistix® and by the α keto acid test (9) The urinary excretion of *o* hydroxy phenylacetic acid and phenylacetylglutamine was determined by paper chromatography (4)

RESULTS

Plasma phenylalanine level during fasting In neither of the patients the plasma phenylalanine level decreased during fasting (Fig 3) Instead there was a slight increase amounting to 0 3 mg/100 ml during 4 hours (1% increase

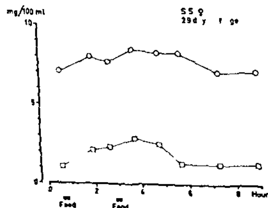


Fig 4 The influence of food on the plasma levels of phenylalanine (—c) and leucine (□—□) in patient S S Zero time corresponds to 8 00 a.m Meals were given at 2 o clock in the night and at the times indicated in the figure Each meal was calculated to contain [illegible]0 mg of phenylalanine

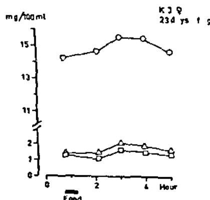


Fig 5 The influence of food on the plasma levels of phenylalanine (o—o) tyrosine (Δ—Δ) and leucine (□—□) in patient K J Zero time corresponds to 10 00 a.m Meals were given at 2 o clock in the night and at the time indicated in the figure The meal was calculated to contain 50 mg of phenylalanine

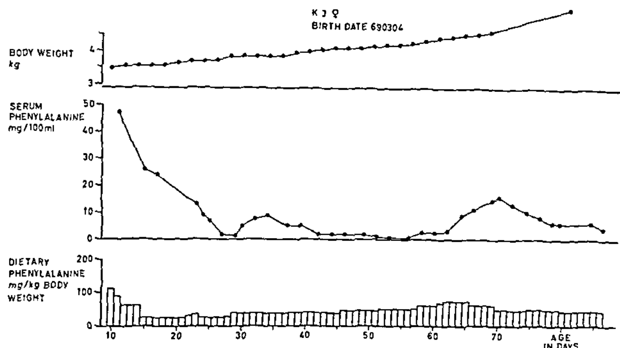


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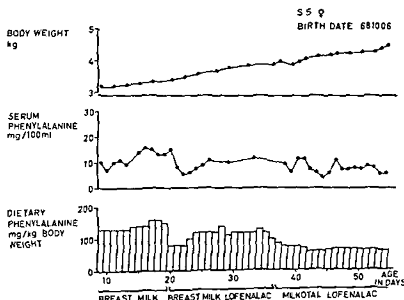


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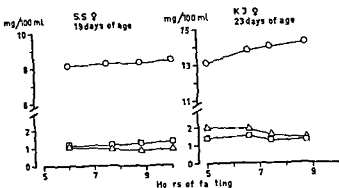


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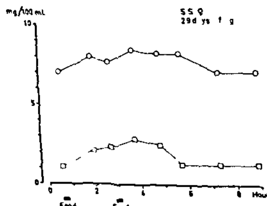


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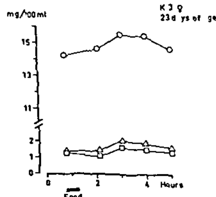


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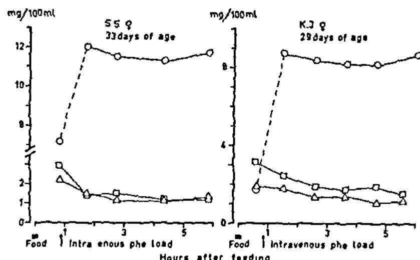


Fig 6 Changes in the plasma levels of phenylalanine (o—o) tyrosine (Δ—Δ) and leucine (□—□) after an intravenous single injection of phenylalanine S S was given 40 mg and K J 53 mg per kg body weight The loading was performed 45 min after the second meal of the day

per hour) in patient S S and 1 1 mg/100 ml (2% per hour) during 4 hours in patient K J During the fasting period the urinary loss of phenylalanine was less than 0 1 mg per hour in both patients The phenylalanine loss by the urine in patient S S was less than 5 mg per day even when the plasma level was at about 15 mg/100 ml

Changes in the plasma phenylalanine level after feeding In patient S S the change in the plasma phenylalanine level after feeding was followed from 8 30 a m to 4 30 p m (Fig 4) Before the study the child was given the ordinary night meal at 2 00 a m During the study two ordinary meals of breast milk were given at 8 40 and 10 55 a m respectively Each meal was calculated to contain 70 mg of phenylalanine There was a slight increase in the phenylalanine level of about 1 mg per 100 ml plasma after each meal with a slow return to the fasting value The fasting level obtained at 8 30 in the morning was reached 4 hours after the second meal In patient K J the plasma phenylalanine level was followed after one of the meals (Fig 5) containing about 50 mg of phenylalanine The changes observed were similar to those in patient S S

Intravenous phenylalanine loading The loading was performed 45 min after the second meal of the day by a rapid infusion of a 2 5% solution of L phenylalanine (kindly supplied by AB Astra Sodertalje) into a scalp vein The increase in the plasma phenylalanine level from 7 2 to 12 0 mg/100 ml (150 mg phenylalanine injected) after 1 hour in patient S S and from 1 7 to 8 7 mg/100 ml (200 mg phenylalanine injected) in patient K J (Fig 6) corresponded to an apparent distribution volume of 3 1 l (body weight 3 82 kg) and 2 9 l (body weight 3 80 kg) respectively

The plasma disappearance rate calculated from the decrease in the plasma phenylalanine level between 2 and 3 hours after the loading i e about 3 to 4 hours after the last meal, was in both patients less than 0 2 mg/100 ml per hour As in the previous tests the plasma phenylalanine level thereafter increased The tyrosine level did not increase after the loading in either of the infants

DISCUSSION

The diagnosis of classical severe PKU is generally based on the following criteria (5 11) a normal phenylalanine level at birth increasing to 30 mg/100 ml or higher at the end of the first week of life but without increase in the tyrosine level the presence of o hydroxy phenylacetic acid and later phenylpyruvic acid in the urine A complete lack of phenylalanine hydroxylase activity has been noted in patients on whom liver biopsy has been performed (10 13 14 15 17 23) In addition to this severe form of PKU other apparently genetic variants of hyperphenylalaninemia are described (3 10, 12, 16, 18 24) Classical mild PKU also

called atypical PKU is characterized by a slow rise in the plasma phenylalanine level and a late appearance of phenylpyruvic aciduria. Dietary treatment is considered necessary. Another variant called persistent hyperphenylalaninemia is generally not accompanied with phenylpyruvic aciduria because the plasma phenylalanine level is only moderately elevated. Mental retardation and other neurological symptoms are considered not to occur (24). In three patients considered to have persistent hyperphenylalaninemia a marked decrease but not an absence of phenylalanine hydroxylase activity have been reported (10, 14). In 1 case with a mild form of classical PKU no activity of the enzyme was observed (14). The reason for the difference in the dietary phenylalanine tolerance in severe and mild PKU is unknown. Oral phenylalanine tolerance tests do not seem to be suitable for differentiating between the two forms of hyperphenylalaninemia (3, 7).

Of the 2 cases studied by us patient K. J. no doubt suffered from severe classical PKU. Patient S. S. had a greater dietary tolerance for phenylalanine and could either be viewed as having a mild form of classical PKU or persistent hyperphenylalaninemia. The blood phenylalanine level never exceeded 16 mg per 100 ml in spite of the fact that the girl for the first 20 days of life was fed breast milk ad libitum. Phenylpyruvic acid was not detected in the urine and o-hydroxyphenylacetic acid only after phenylalanine loading. This difference in the dietary phenylalanine tolerance between the two infants is most easily explained assuming a persistent phenylalanine hydroxylase activity in patient S. S. Patient K. J.—as a case of classical severe PKU—was considered to have a complete lack of enzyme activity. However the results obtained gave no definite evidence for a persistent phenylalanine hydroxylase activity in patient S. S. The changes of the phenylalanine levels during fasting after ordinary meals and after intravenous phenylalanine loadings were similar in the two patients. The only difference observed was an insignificantly greater increase in the plasma phenylalanine level during fasting in patient K. J. (Fig. 3). We have not had the opportunity to perform direct measurements of the enzyme activity on a liver biopsy. The fact that the plasma phenylalanine level increased during fasting shows that the excretion and metabolism of phenylalanine was less than the amount of phenylalanine liberated by breakdown of protein during the catabolic phase. The results obtained emphasize the difficulties in getting relevant information from the plasma disappearance rate of an amino acid even after intravenous infusions as infants when fasting soon turns into a catabolic state.

The clear increase in the plasma phenylalanine after milk feeding (Figs. 4 and 5) and the fact that the urinary excretion of phenylalanine was small make a defect intestinal absorption or a reduced renal tubular reabsorption improbable as the cause of the greater dietary phenylalanine tolerance in patient S. S.

Mutation of a structure gene causes a change in the structure of a specific protein. If this protein is an enzyme the result can be either a partial or a complete loss of enzyme activity. Such a genetic modification of an enzyme molecule can change its K_m value. A high K_m value of the phenylalanine hydroxylase in patient S. S. might explain why the plasma phenylalanine level did not rise to the excessive high values seen in severe classical PKU. We have not been able to test this hypothesis as the family has moved to another part of Sweden. The fact that the phenylalanine intake had to be gradually reduced to about 40 mg per kg and day when the child was 3 months old to reach a suitable plasma phenylalanine level is not inconsistent with this hypothesis.

As mentioned above there are no doubt several genetic variants of hyperphenylalaninemia. Kinetic studies of the enzyme with determinations of K_m and V_{max} and probably also genetic studies have to be made to characterize the different forms of hyperphenylalaninemia. Such studies have not yet been performed. Therefore we have to rely on more empirical

classifications According to the present classification proposed by WHO (24) the atypical case S S ought to be viewed as having a mild form of classical PKU Diet therapy was considered necessary with regard to the present minor knowledge about the causes of mental retardation in PKU

SUMMARY

Two infants with hyperphenylalaninemia first recognized at the age of 5 days were studied One of the patients was a case of classical severe PKU The other patient, on an unrestricted diet, showed only a slow rise in the plasma phenylalanine level amounting to 16 mg/100 ml at 3 weeks of age and had no phenylpyruvic aciduria The plasma phenylalanine levels of the two infants were determined during fasting, after feeding and after intravenous phenylalanine loading The results obtained did not reveal any significant difference in the phenylalanine turn over between the two infants

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The human foetal red cell is different from that of the adult because of its surface morphology (16) ultrastructure (33) size (17) mechanical (5) and osmotic fragility (7 8 31 34 43 45) heat (3 9) and acid resistance (22) The membrane permeability is also different and in foetal red cells the thiourea and the glycerol have a slow penetration (14 15) the water has a slow diffusion (1) and the glucose shows a greater permeability (47) However the rate of lysis in hypotonic solutions of glucose is similar in foetal and in adult red cells (40)

The foetal red cell contains predominantly haemoglobin F a lower amount of acetyl cholinesterase (14 21 26) and a higher glucose 6 phosphate dehydrogenase activity (26 38) and it shows a different electrophoretic mobility (14 37)

The activities of carbonic anydrase (44) of catalase (20 26) of phosphofructokinase (6 25 30) and of NADH methemoglobin reductase (24 25 35 36) are reduced so as a relative deficiency of membrane adenosine triphosphatase (46) is present Pyruvate kinase (26) lactic dehydrogenase (26) fructose 1 6-diphosphate aldolase (26) enolase (30) phosphoglycerokinase (30) activities are increased The glycolytic intermediates phosphoenolpyruvate and 2–3 diphosphoglycerate are reduced (30) The levels of ATP and glucose consumption are higher (29) and ATP and glutathione instabilities are greater (29) in newborn red cells

The erythrocytes of newborn infants lose potassium at an accelerated rate when stored at 4 C or when incubated at 37 C (4 43) for a decreased active potassium influx (4)

Foetal cells show a higher reactivity when tested with antigloboside and anti hematoside serum (2)

The characteristics of the foetal red cells are not yet fully evaluated especially in order to the period of life when the functional maturation is complete

Acis lysis of red cells is probably a very indicative index of chemical changes in the membrane accompanying ageing red cells (10) and of its water content (13 28)

In view of this problem acid lysis of the foetal and adult erythrocytes was studied by means of an automated procedure

MATERIALS AND METHODS

Capillary blood obtained by finger prick from full term newborns aged from 1 to 6 days from infants aged from 7 to 360 days and from children aged from 2 to 10 years was used All the subjects were healthy

The samples of blood were diluted 1 30 (20 μl of blood and 6 0 ml of isotonic NaCl solution buffered to pH 7 3 by veronal HCl)

Acid haemolysis was obtained by submitting the red blood cell suspension to a progressive increasing hydrogen ion concentration by dialysis against an isotonic solution of low pH (10)

The degree of haemolysis as a function of continuously reduced pH was automatically recorded by means of the Fragiligraph (model D 2 Elron Electronic Ind Haifa, Israel)

A sample of 40 μl of the red cell suspension was introduced into a microcuvette of 0 04 ml capacity

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day of life is augmented ($p < 0\ 01$) The time of acid lysis which is more reduced between 2 and 3 days of life increases between 4 and 6 days of life and still augments in the second week of life The differences are very significant ($p = 0\ 001$)

Sub equently the time of acid lysis shows a progressive decrease and the values are similar to the normal values after the ninetieth day of life

The increased resistance of red cells to acid lysis and the decrea e of the time of lysis in creasing age are similar for the initial and for 50° and 100

The rate of haemolysis expressed as pe cent per minute is different ($p = 0\ 01$) only in infants aged from 2 to 3 days and from the second week of life The rate of lysis is normal after the sixtieth day of life

Table 2 *Statistical analysis (t test of Student) of time of acid lysis of red blood cells obtained in infants versus normal children*

p = probability N S = not significant

Age (days)	Acid haemolysis 0*	50	100	Rate of haemolysis
1	$p < 0\ 01$	$< 0\ 01$	$< 0\ 01$	NS
2–3	$p < 0\ 01$	$< 0\ 01$	$< 0\ 01$	$< 0\ 01$
4–6	$p < 0\ 01$	$< 0\ 01$	$< 0\ 01$	NS
7–15	$p < 0\ 01$	$< 0\ 01$	$< 0\ 01$	$< 0\ 01$
16–30	$p < 0\ 01$	0 01	$< 0\ 01$	$> 0\ 01$
31–60	p 0 01	$< 0\ 01$	$< 0\ 01$	$< 0\ 01$
61–90	$p < 0\ 01$	$< 0\ 01$	0 01	NS
91–180	p NS	NS	NS	NS
181–360	p NS	NS	NS	NS
2nd year	p NS	NS	NS	NS
1 day versus 2 3 days	p 0 01	$< 0\ 01$	0 01	$< 0\ 01$
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DISCUSSION

Minimal changes in pH alter the osmotic frag ility of erythrocytes (19 31) The influence of pH has been attributed to changes both in the osmotic properties of haemoglobin and in the binding of cations by the haemoglobin (19)

The increase in the osmotic fragility of erythrocytes at lower pH values is the result of changes in cell water and in volume for an increase in the osmotic activity in the cell (13 27 28) The decrease of pH is associated with a relative increase in solvent water and a relative decrea e in "bound water but the mechanism whereby pH influences the *amount* of water involved in osmotic equilibrium is not known (27 28)

It has been suggested (12) that the change in osmotic properties of cell water at different pH levels results from changes in the effective charge on the hemoglobin molecule with dilu tion (28) Alterations in the relative content of Na and K and changes in temperature from 5°C to 37 C (28) does not influence the os motic behaviour of cell water

The present study shows that the foetal red cells are different from the adult ones with re gard to the acid lysis and they are more resist ant to the osmotic lysis when the pH of the me dium decreases This characteristic is more marked after 2 or 3 days of life and in the sec ond week of life The resistance to the acid ly sis decreases progressively and reaches the val ues similar to that of the older children after 90 days of life The normalization of the acid lysis is not related to the behaviour of the red cells to the hypotonic saline solutions (41) because the normal hypotonic lysis is reached in the second year of life (41)

The resistance to the acid lysis can be re lated to the amount of foetal haemoglobin that shows a gradual decline during the first weeks of life and a more rapid decline at about 2 months of age terminating in levels of about 10 at 4 months of age (11) But we have observed that the acid lysis is diminished both in red cells from thalassemia major and in the younger red cells from thalassemia trait with high and respectively low levels of haemoglo bin F(42)

Moreover the loss of K of the foetal red cells does not influence the acid lysis (4 28)

The behaviour of the acid lysis is related however to the shortened life span of the foe

Table 1 *Time of acid lysis of red blood cells expressed as mean values ± S D*

Age (days)	Subjects (number)	Acid haemolysis (seconds) 0	50	100 *	Rate of haemolysis (/minute)
1	8	181±40	238±53	416±69	49 0±14 9
2-3	11	242±36	326±37	510±43	30 8± 6 2
4-6	8	190±23	272±33	450±73	43 5±13 0
7-15	10	220±33	307±27	494±52	36 5± 5 7
16-30	10	189±29	274±31	453±49	41 8± 4 6
31-60	9	165±19	247±21	436±80	32 9±13 0
61-90	10	151± 7	227±19	374±19	43 7± 9 7
91-180	16	140±17	208±23	335±74	50 2± 9 3
181-360	16	138±24	216±25	330±37	47 3±12 9
2nd year	10	132±15	209±18	323±20	48 3±10 0
6-10 years	12	133± 9	209± 6	320±29	46 4± 5 9

and of 0 5 mm path two walls of which are made of dialysing membrane The microcuvette was held in a bath containing isotonic NaCl solution adjusted to pH 2 45 by HCl at thermostatically controlled temperature of 25 C

The gradual increase of the number of red cells haemolysed is resulted in a progressive increase in the light transmission through the cell suspension and provides a cumulative and a derivative curve to reduced pH

Younger cells are most sensitive to acid lysis in contrast to osmotic lysis in which the old cells are the first to lyse (10)

The results are expressed as lysis in function of time and the following measurements are

(*a*) time in seconds required from the start of the curve to the initial lysis and to 50 and 100 of lysis

(*b*) slope of the curve between 25 and 75 of lysis and calculation of the rate of haemolysis as per cent/minute

Statistical analysis are determined with the calculation of the Standard Deviation (S D) and of the *t* test of Student

RESULTS

The results of the acid lysis of red blood cells in normal children aged from 1 day to 10 years are compiled in Table 1 and in Fig 1

Statistical analysis were determined on the results of the infants versus the results of normal children aged from 6 to 10 years (Table 2)

The time of red cell acid lysis in the first

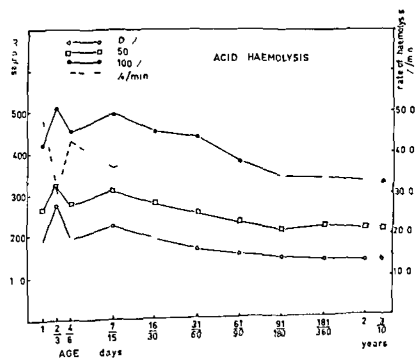


Fig 1 Time of acid lysis of red cells (mean values) in relation to the increase of the age

day of life is augmented ($p<0\ 01$) The time of acid lysis which is more reduced between 2 and 3 days of life increases between 4 and 6 days of life and still augments in the second week of life The differences are very significant ($p=0\ 001$)

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DISCUSSION

Minimal changes in pH alter the osmotic fragility of erythrocytes (19 31) The influence of pH has been attributed to changes both in the osmotic properties of haemoglobin and in the binding of cations by the haemoglobin (19)

The increase in the osmotic fragility of erythrocytes at lower pH values is the result of changes in cell water and in volume for an increase in the osmotic activity in the cell (13 27 28) The decrease of pH is associated with a relative increase in "solvent water and a relative decrea e in bound water but the mechanism whereby pH influences the amount of water involved in osmotic equilibrium is not known (27 28)

It has been suggested (12) that the change in osmotic properties of cell water at different pH levels results from changes in the effective charge on the hemoglobin molecule with dilution (28) Alterations in the relative content of Na and K and changes in temperature from 5 C to 37 C (28) does not influence the osmotic behaviour of cell water

The present study shows that the foetal red cells are different from the adult ones with regard to the acid lysis and they are more resistant to the osmotic lysis when the pH of the medium decreases This characteristic is more marked after 2 or 3 days of life and in the second week of life The resistance to the acid lysis decreases progressively and reaches the values similar to that of the older children after 90 days of life The normalization of the acid lysis is not related to the behaviour of the red cells to the hypotonic saline solutions (41) because the normal hypotonic lysis is reached in the second year of life (41)

The resistance to the acid lysis can be related to the amount of foetal haemoglobin that show a gradual decline during the first weeks of life and a more rapid decline at about 2 months of age terminating in levels of about 10 at 4 months of age (11) But we have observed that the acid lysis is diminished both in red cells from thalassemia major and in the younger red cells from thalassemia trait with high and respectively low levels of haemoglobin F(42)

Moreover the loss of K of the foetal red cells does not influence the acid lysis (4 28)

The behaviour of the acid lysis is related however to the shortened life span of the foe

tal erythrocytes The life span has been demonstrated as 80 days (32) from 56 to 105 days (48) from 70 to 80 days (23) from 82 to 131 days (18), as 83 days (11) and it is very possible about 90 days

The progressive reduction of the resistance to the acid lysis is related to the disappearance of the foetal red cells and to the presence of adult type of red cells The slow diffusion of water in the foetal red cells (1) prevents the rapid increase of the red cell water when the pH falls and delays the lysis

SUMMARY

Acid lysis of foetal red cells has been investigated by means of an automated procedure with the Fragiligraph

The time of red cells acid lysis is augmented in the first weeks of life and returns normal after the ninetieth day of life

The rate of acid lysis is normal only after the sixtieth day of life

The behaviour of the acid lysis is related to the disappearance of the foetal erythrocytes and to the presence of adult type of red cells

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L ASPARAGINASE TREATMENT OF ACUTE LEUKAEMIA IN CHILDREN

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L asparaginase, an enzyme which is found in guinea pig serum, turned out to be active against certain neoplasms in rodents (4, 8) This enzyme can also be extracted from *E coli* cultures (1) Oettgen, Haskell, McElwain & Hill (6, 7, 12, 13) proved it to be effective in the treatment of leukaemia in man

The enzyme breaks down asparagine to aspartic acid cells which lack an asparagine synthesizing system can develop a lethal deficiency of this amino acid (2, 5)

It was formerly thought that in leukaemia only the asparagine dependent blasts would suffer and that other blood and bone marrow cells were not damaged but it has been shown (1, 11) that lymphocytes function normally only in the presence of asparagine, and perhaps lack a synthesizing system

Some information on the results including the course of the disease and some biochemical data are given in this article

PATIENTS AND METHODS

L asparaginase was usually given intravenously daily in a dosage of 200 U/kg bwt The injection lasted a few minutes extravasation gave little or no irritation In the first patients it was given alone but later on it was combined with prenisone 80-100 mg/m² body surface Bone marrow punctures were done at the start after 14 and 28 days of therapy The number of children treated was 18 of whom 4 were in a relapse and 14 had had no prior treatment One of them had a monocytomonoblastic type of leukaemia One formerly untreated child is discussed separately because his cell type (case 18 D K) was very unusual he is not mentioned in the table of untreated cases

Particulars are shown in Table I The remaining twelve were considered to have the lymphoblastic type of leukaemia

In the untreated cases asparaginase therapy had to be stopped twice in case 7 because of an anaphylactic shock

In case 13 the bone marrow was severely hypocellular After cessation of asparaginase treatment prednisone was continued and the bonemarrow showed a repopulation of normal elements again

RESULTS

Bone-marrow and peripheral blood

If the definition Complete remission" implies the presence of a normocellular bone marrow then full remissions were not so numerous (see Table I) The hypocellularity makes it also rather illusory to say with reasonable certainty whether blastcells are present or absent Another difficulty not mentioned elsewhere was the relatively high number of lymphocytes or lymphocyte like cells often found in these bone marrow smears

In patients number 1 2 10 12 and 13 a hypocellular bone-marrow was found at the end of the treatment with signs of recovering erythro and granulopoiesis Blastcells were, with the reservations mentioned above considered to be absent As treatment was continued with cytostatics it cannot be claimed

Table 1 *Patients with no prior treatment*

Patients	Sex	Age	Type of leucaemia	Duration of therapy (days) Aspara ginase	Predni sone	Findings after stopping asparaginase Bone marrow Cellularity	Blasts	Peripheral blood Hb (g/100 ml)	Leucocytes (per mm³)	Platelets (per mm³)
1 R S	o	1 0	A Mo L	28	—	Hypocellular	Absent	9 5	5 600	241 000
2 M G	♀	1 1	A L L	28	28	Hypocellular	Absent	11 9	5 700	107 000
3 J D	♂	2 1	A L L	28	10	Normal	5	11 5	3 000	71 000
4 H B	♂	3 3	A L L	28	28	Normal	Absent	9 9	3 400	121 000
5 F L	♀	3 6	A L L	14	14	Normal	90	7 4	1 400	28 000
6 G M	♂	3 9	A L L	21	10	Normal	40*	9 8	13 600 + blasts	8 000
7 P R	♂	4 3	A L L	10	10	Normal	30*	9 0	2 160	10 000
8 F V	♂	4 10	A L L	28	28	Normal	Absent	11 6	7 600	228 000
9 M G	♀	6 8	A L L	28	28	Normal	Absent	14 2	4 200	139 000
10 E S	o	7 0	A L L	21	28	Hypocellular	Absent	12 5	5 500	101 000
11 R H	o	7 1	A L L	28	—	Normal	Absent	10 1	5 100	230 000
12 Y B	♂	8 2	A L L	28	28	Hypocellular	Absent	11 3	2 800	130 000
13 H H	♀	9 5	A L L	19	19	Hypocellular	Absent	11 9	1 700	40 000

that this therapy alone resulted in full remissions A partial remission was obtained in patient 3

In the peripheral bloodcell-counts it was found that if the marrow was hypocellular a rise toward normal numbers of erythrocytes granulocytes and platelets was seen However only two of these 5 patients had obtained a normal number of all the three elements The others had subnormal numbers of one or two of the three The platelets were more than 100 000/mm³ in 4 patients but in one they were still subnormal but within the safe range (40 000) In patients 4 8 9 and 11 the bone marrow was normocellular and no blastcells were detectable The peripheral blood was normal in these four patients except for a subnormal haemoglobin content in case 4

In the case of patient 6 asparaginase was stopped after 21 days the bone marrow still contained more than 40% blasts and a recommencing erythropoiesis was seen

Patients in relapse

Four patients in relapse were treated in two of them we believe it had no beneficial effect Patient 14 (J J) a 14 year old boy had an acute myeloblastic leukaemia Despite intense cytostatic drug treatment a complete remission was not obtained after 8 months Only a very partial remission not long lasting was the best result in this boy Peripheral bloodmyeloblasts turned out to be very sensitive *in vitro* for asparaginase After starting treatment the number of blasts dropped from 56 800 to 9 000/mm³ in 1 week Thereafter there was a rise in the next 10 days

Patient 15 (M R) was a 9 year-old girl suffering from acute myeloblastic leukaemia She had been in a continuous remission for 13 months when a relapse developed Various cytostatics resulted only in a very temporary partial effect Myeloblasts from bone marrow proved to be very sensitive *in vitro* for asparaginase The patient was treated with asparaginase 200 U/kg bwt daily for 6 days and as the number of blasts in the peripheral blood did not drop the dosage was doubled for an other 7 days without success The father wanted her to be home again and as we felt there was no real hope for a remission we could not but agree to his wish Treatment was stopped she died at home An autopsy was not performed

Patient 16 was a 10 year-old girl (M V) When she was 8 years old a diagnosis of acute leukaemia was made elsewhere and she was treated successfully Later the initial diagnosis

Table 2 *Correlation between the test of leukaemic cells for L-asparaginase requirement in vitro and clinical success of L asparaginase therapy*

Patients	Type of leukaemia	Percentage ^3H uridine incorporation into nucleic acids			Clinical success
		+L asparagine	−L asparagine	+L asparagine	
1 R S	A Mo L	100	58	31	+
5 F L	A L L	100	78	47	−
7 P R	A L L	100	60	44	+
8 F V	A L L	100	63	76	+
9 M G	A L L	100	97	78	+
10 E S	A L L	100	109	114	−
12 Y B	A L L	100	88	72	+
14 J J	A My L	100	78	32	−
15 M R	A My L	100	70	26	−
17 A T	A My L	100	99	76	+
18 D K	A Myeloegranuloc L	100	82	41	−

was doubted and all treatment was stopped Her condition remained excellent for about 10 months when a relapse occurred In that stage she was admitted to our hospital Treatment was started with prednisone and asparaginase 200 U/kg bwt daily She developed a high temperature with extreme vomiting and an extreme tenderness of the abdomen Asparaginase treatment was discontinued An *E coli* sepsis was diagnosed and she died 11 days after the beginning of therapy, and 6 days after stopping asparaginase therapy The clinical picture was suggestive for an acute pancreatitis At autopsy however the pancreas proved to be normal Patient 17 a 8 year old boy with a fourth relapse of his acute myeloblastic leukaemia had become resistant to the usual cytostatics and prednisone As our results in the other three patients with 200 U/kg bwt were not satisfying the dose in this patient was arbitrarily, set at 500 U/kg bwt given intramuscularly After 28 days a full remission was obtained

Acute myelocytic granulocytic leukaemia

Patient 18 was a 9 year old boy (D K) suffering from an acute myelocytic granulocytic leukaemia In culture his cells seemed to be very sensitive and he was treated with L-asparaginase alone (200 U/kg bwt) The blasts in the blood disappeared after a few days but only to rise again a few days later Trial with a higher dosage was not done

Infection during treatment

Only one patient (case 16 M V) died from an infection (*E coli* sepsis) The general impression was that infections were rarer and less severe than in our other unreported series of patients treated with rubidomycine vincristine and prednisone

A *staphylococcus aureus* sepsis developed in one patient (case 13 H H) during the course of treatment she recovered with antibiotics and incision of an abscess

In patient 1 treatment with asparaginase was started when he was still suffering from a furunculosis Our impression was that this therapy did not interfere with the recovery from that infection

Complications of the treatment

Allergic reactions As asparaginase is a bacterial protein it could give rise to immunological allergic reactions They have been described e g by Hill et al (7) It is also possible that an allergy to this *E coli* product exists already before the commencement of therapy Therefore a pretreatment-test is done as a routine by injecting 10 I U intracutaneously The reaction is read 2 hours later In all our patients this was done and an allergic like reac-

tion was never found None the less one patient (case 7 P R) developed an anaphylactic shock in a few minutes after completing the injection This occurred on the tenth day of treatment The symptoms subsided after a few hours

Liver disorders Serum alkaline phosphatase thymol turbidity and serum bilirubin were always normal Slight transient rises of SGOT and SGPT were noticed One child developed a severely disturbed liver function one week after stopping asparaginase Total serum protein also tended to fall There was a selective fall in the albumin level though not great enough to cause oedema

Kidney function The slight increase of bloodurea by 100–400 mg/litre was rather constant Creatinine bloodlevels remained unchanged and proteinurea or urinary sediment abnormalities were not found

Coagulation disorders In all the patients a drop of serumfibrinogen was seen It has been demonstrated that this fall is due to decreased synthesis of fibrinogen (3) Other coagulation factors were not investigated We cannot state that more haemorrhages occurred than with other forms of treatment

Uric acid metabolism Blood levels remained normal the urinary excretion was initially high but dropped to normal values during treatment Allopurinol was not used renal colic or other signs of stone formation was not observed

Further toxicity Gastrointestinal symptoms were slight or absent neurological toxicity was not observed In our opinion this drug is usually well tolerated

Correlation between in vitro test and clinical effectiveness

The test for L asparagine requirement was performed with cell suspensions from the bone marrow or from the buffy coat (13) This assay involves measuring the incorporation of ^{3}H uridine into nucleic acids by cells incubated in the presence and absence of L asparagine and in the presence of L asparaginase respectively In Table 2 for 11 from 18 patie scribed above the correlation is given betv the L asparagine dependency of the leukaem cells *in vitro* and the response of the patients to L asparaginase

The results given in Table 2 show that in 6 out of 11 cases (patients 9 10 14 15 17 18) there was a reverse correlation between in vitro results and the effect of clinical treatment

CONCLUSION

L-asparaginase may be used for the initial traetment of acute leukaemia The remission rate after four weeks of treatment does not seem to be higher than that obtained with combinations of cytostatics and prednisone If looked for asparaginase gives rise to many biochemical abnormalities which turn out to be reversible Fibrinogenopenia ought to be a real disadvantage but in our experience we felt it did not cause many difficulties

L asparaginase is usually well tolerated Lifethreatening allergic manifestations may however occur rather unexpectedly

The results in the four children with relapse were disappointing though three of them were of the myeloblastic type which is less sensitive to most types of therapy than the lymphoblastic type

The value of the *in vitro* asparaginase sensitivity test is poor a hopeful outcome may be followed by an *in vivo* failure or vice versa The dosage of 200 U/kg bwt seemed to be very satisfying If the blastcells are not asparagine-dependent or if they can easily develop the asparagine synthesizing system a higher dosage scheme would not work either Therefore we doubt if the very high dosages used by others (10 12) are a real advantage Toxicity surely increases with increased dosage effectiveness is another question

If our results are compared with Mathe et al (10) the remission rate is higher in this series of patients We presume that this was due to the combination treatment with prednisone because their patients were treated with L-aspara

gınase only but ın much hıgher dosage 400 to 800 U/kg bwt daılv ın 2 dıvıded doses On the other hand McElwaın & Hardısty (12) gave 1 000 U/kg bwt but alone, to 9 chıldren wıth acute lymphoblastıc leukaemıa ın relapse After pretreatment wıth cytosınearabınosıde, the blasts ın the bone-marrow varıed from 99 to 11% Thıs was followed ımmedıately wıth asparagınase only for 14 to 28 days Full remıssıons were obtaıned ın 8 out of 9 patıents

SUMMARY

The results of asparagınase prednısone therapy ın chıldren are reported In 12 chıldren wıth untreated acute lymphoblastıc leukaemıa 9 remıssıons were obtaıned, there were 2 faılures and ın one patıent therapy had to be stopped because of an anaphylactıc shock

One chıld wıth an acute myelocytıc granulocytıc type of acute leukaemıa dıd not respond One chıld wıth an acute monocytıc monoblastıc leukaemıa was gıven asparagınase only He responded very well

Four patıents wıth a relapse of an acute myeloblastıc leukaemıa were treated there were two faılures one chıld dıed too early from an ınfectıon to be evaluated One chıld showed a very good reactıon Serıous sıde effects were few, the drugs gave many bıochemıcal dısturbances ıncludıng a constant hypofıbrınogenaemıa but these were well tolerated and reversıble

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SEPARATION BY GEL FILTRATION AND MICRODETERMINATION OF UNBOUND BILIRUBIN

I In Vitro Albumin and Acidosis Effects on Albumin Bilirubin Binding

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It is now possible to separate unbound bili rubin from serum either by dialysis (16) $CaCO_3$ adsorption (25) or gel filtration (5 8 9 10 27) As the unbound bilirubin serum concentration is low we adapted in our study an extraction technique on Sephadex micro-columns and a diazoreaction method in order to analyze the small serum volumes usually drawn from the newborn infants

Albumin and pH effect on albumin bilirubin binding has been studied on newborn sera as well as on commercial bilirubin and human albumin mixtures

MATERIAL AND METHODS

Pe liatric Versatol (Warner-Chilcott Laboratories)

Bilirubin (Calbiochem) dissolved in Na CO 0 1 M just before use Human albumin (Centre National de Transfusion Sanguine—Paris) 78% purity dissolved in PO buffer 0 1 M

Sephadex G 25 Medium (Pharmacia Uppsala Sweden) suspended in PO buffer 0 1 M

Serum from icteric premature or full term infants submitted to exchange transfusion for erythroblastosis foetalis

Manipulations take place at room temperature (? – ?4 C) in half light A fine catheter closed with a grip is adjusted to the lower extremity of 5 ml pipettes (0 6 cm diameter 0 1 ml graduations) cut at ?0 cm height so that any volume added with Pasteur pipettes on the column or collected in analysis tubes can be directly appreciated from the column graduations In order to lessen the dilution of the samples on the column the Sephadex bed fills only 2 3 ml on the column graduations The 0 5 ml sample is layered over the Sephadex The flow is stopped and the walls are washed with 0 3 ml PO buffer 0 1 M When a thin layer just covers the Sephadex bed the outlet is closed and 1.5 ml PO buffer is added and the first 1 5 ml of eluant is collected (Peak 1" con taining all the proteins of the sample and all bili rubin bound to albumin) The column is washed with 2 ml buffer In order to trap all unbound bilirubin left on the Sephadex 0 5 ml albumin solution (10 mg/ml in PO buffer 0 1 M pH 7 4) is added and allowed to penetrate almost completely into the S phadex The flow is stopped and the column is filled with eluant buffer 30 min later the outlet is opened again after 0 3 ml buffer has drained into the bed 1 ml eluant is collected (Peak 2" con taining all human albumin added and bilirubin pre viously left on the column)

When washed with 5 to 10 ml buffer th same column can b used repeatedly A well trained person can p rform separation and bilirubin determination of one serum within one hour If one works with 4 columns it is possible to analy e 4 samples in 2 hours

For the unbound bilirubin *determination* the Michaelsson technique (18) sensitivity has been in creased by concentrating several reagents in order to obtain a coloration of adequate intensity in a re duced volume

Sample	0 25 ml
Diphylin	0 20 ml (unmodified reagent)
Diazoreagents	0 10 ml (2 5 × concentrated *reagent*)
Ascorbic acid	0 01 ml (5 × concentrated reagent)
Fehling	0 15 ml (unmodified reagent)
Final volume	0 71 ml

Abbreviations PAG polyacrylamide gel PSP phenolsulfonphtalein dye HBABA 2-(4 hydroxy benzeneazo) benzoic acid In this study unbound bili rubin is assumed to be unconjugated

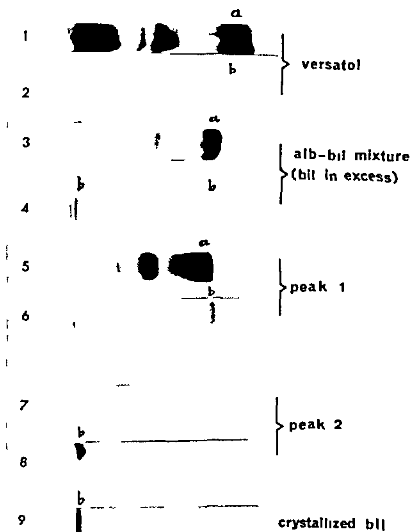


Fig 1 Identification of bound and unbound bilirubin by PAG electrophoresis Protein bands move from left to right and are located with 1 (w/v) Amido black in 7 % (v/v) acetic acid (gels 1 3 5 7) Yellow bilirubin spots are visible on unstained gels (gels 2 4 6 8 and 9) a albumin b bilirubin x peaks 1 and 2 from Sephadex column In this particular case peak 2 is not extracted with an albumin solution but with Na CO 0 1 M xx crystallized bilirubin is dissolved in Na CO 0 1 M

Reading is done at 600 μm on a Jobin Yvon spectrophotometer in pyrex microcuves (25×10×2 mm)

Pediatric Versatol is used for the standard curve the assay was linear within the 0 1 to 2 0 μg interval

Two different amounts of bilirubin assayed in the same conditions gave respectively 0 79 μg±0 06 μg and 1 73 μg±0 06 μg (mean±1 SD n=10 in each case)

RESULTS

Gel filtration Separation on Sephadex column chromatography and identification of both bilirubin types are shown on Fig 1 On PAG electrophoresis (12) pure bilirubin or unbound bilirubin stays at the origin whereas bound bilirubin migrates with albumin

In experiments using either pure bilirubin solution, serum or albumin-bilirubin mixtures containing *one or* both bilirubin types the total bilirubin recovered after gel filtration was 97 7% ±2 9% n=14 (mean±1 SD)

In the same conditions each of 10 samples were analysed on two different columns and the difference observed between each column in terms of total bilirubin recovered was 1 35%2 05% ± (mean ± 1 SD)

pH and albumin concentration effects A constant bilirubin amount is added on columns conditioned at different pHs The column is loaded with an excess albumin solution (pH identical to the pH of the column) in order to pick up bilirubin

The relation existing between bilirubin attachment and pH could be apparent and is actually due to a decrease of bilirubin solubility in water when the pH is lowered (1) As a matter of fact when albumin and bilirubin are mixed in identical conditions of pH and

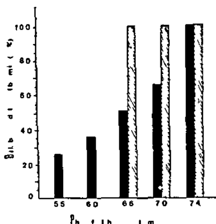


Fig 2 Bilirubin bound to albumin at different pH s Sephadex G 25 columns are conditioned with PO buffer 0 1 M at pH s 5 5 6 0 6 6 7 0 and 7 4 After addition of 25 μg bilirubin (dissolved in Na_2CO_3 0 1 M) the columns are washed with the corresponding buffer Albumin (12 5 mg) dissolved in PO buffer 0 1 M (pH identical to the pH of the column) is added on the column Bound bilirubin (peak 1') is measured and expressed as % of th total bilirubin added on th columns when the binding takes place on the column (dark areas) or in the test tube before gel filtration (shaded areas)

concentration and then added on the column bilirubin is completely bound to albumin what ever th pH might be (Fig 2)

In order to avoid these solubility problems on the column at low pH s albumin and bilirubin are at first mixed in variable proportions and at different pH s (Fig 3)

Bilirubin binding becomes higher and higher as albumin proportions increase in the mixtures Furthermore for a given albumin bilirubin mixture the proportion of protein bound bilirubin increases with the pH

Acidification of icteric sera Only protein bound bilirubin was detected in 4 icteric sera After acidification with PO_4 buffer 0 1 M pH 5 0 unbound bilirubin appeared and the proportion of this fraction seemed to be related to the degree of acidosis (Table 1)

Role of the pH of the column As pH modifies the relative proportions of bound and unbound bilirubin it was important to find out whether the pH of the column should be adjusted each time to that of the serum before being analysed

As seen in Table 2 the equilibrium existing in a sample between both bilirubin fractions is not affected by the pH of the column (at least between pH 7 0 and 7 4) Therefore we use columns systematically conditioned at pH 7 4 for the analysis of newborn infants sera

DISCUSSION

An equilibrium exists in the plasma between the non conjugated unbound bilirubin fraction and the protein bound bilirubin fraction Plasma concentration of unbound bilirubin may represent 1% of the total bilirubin (20) and depends on the serum protein amount (more precisely albumin amount) and on its bilirubin binding capacity (3 10 17 20 27 30) on the acido-basic equilibrium (2 3 13 14 15 16 19 28 33) and on endogenic or exogenic competitive compounds which occupy binding sites on albumin (4 6 11 17 23 29)

It is interesting to know whether in a given serum albumin is still capable of binding additional bilirubin or is already saturated (7 17 20 24 26 31) If unbound bilirubin is toxic it eems however more logical to isolate and determine this fraction and to study

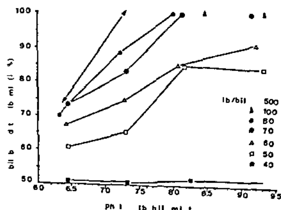


Fig 3 pH effect on bilirubin binding capacity of albumin. Bilirubin (dissolved in Na_2CO_3 0 1 M) is mixed with albumin solution in PO buffer Albumin bilirubin weight ratios as indicated The pH of albumin solutions is chosen according to the final pH desired in the albumin bilirubin mixtures Columns are adjusted to this final pH with PO 0 1 M buffer 25 μg bilirubin are added on the column and bound bilirubin is measured in peak 1"

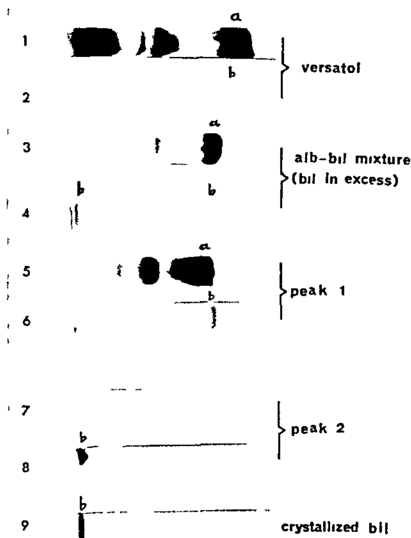


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most serum compounds and competitive endogenic or exogenic substances The effect of such substances as fatty acids α globulins lipoproteins on albumin bilirubin binding has not been thoroughly studied (25 32) and these factors could account for the individual variations in the albumin binding capacity observed with different era (25)

SUMMARY

A gel filtration technique and a diazoreaction method for unbound bilirubin separation and determination directly applicable to small blood volumes has b en described

Precision of the microdetermination reproducibility of the separation method and bilirubin recovery have been very satisfactory in our hands

The effects of pH and relative albumin concentration on the albumin bilirubin binding were studied in sera and in commercial albumin bilirubin mixtures

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Table 1 *Unbound bilirubin appearance in acidified icteric sera*[a]

Before acidification				After acidification			
Total bil conc in s rum (μg/ml)	pH	Bil added on column (μg)	Unbound bil recovered (μg)	pH	Bil added on column (μg)	Unbound bil recovered (μg)	Unbound bil recovered (of total bil)
180	8 94	90	0	6 04	26	1 2	4 6
180	8 94	90	0	6 37	40	1 6	4 0
200	8 55	100	0	6 88	66 6	1 2	1 8
160	8 65	80	0	7 06	61 5	1 0	1 5

[a] Sera previously stored in refrigerator are analysed on Sephadex columns before and after acidification with PO_4 buffer 0 1 M pH 5 0 Bilirubin is determined in serum and in peak 2 before and after acidification

the factors involved in its plasma concentration

With the Rutkowskis technique (25) one can separate unbound from the protein bound bilirubin, unfortunately, we could not recover the unbound bilirubin when it was adsorbed on $CaCO_3$

Sephadex gel filtration has been used by previous authors (*5, 8, 9, 10 27*) and we found *this technique to be adaptable for newborn* icteric serum analysis with good reproducibility and bilirubin recovery

Acidosis increases unbound bilirubin concentration in serum and tissues (20) With sera and albumin bilirubin mixtures we observed that acidosis not only decreases the binding of bilirubin by albumin but also unbinds bilirubin already attached to albumin in a proportion which seems to be in relation to the degree of acidosis

Table 2 *Effect of the initial pH of the column on the equilibrium between unbound and bound bilirubin*[a]

	Peak 1		Peak 2	
pH of the buffer on the column	7 0	7 4	7 0	7 4
Alb /bil weight ratio = 80 Final pH of the mixture 7 0	88 8	88 1	11 2	11 9
Alb /bil weight ratio = 50 Final pH of the mixture 7 4	69 3	68 6	30 7	31 4

[a] Bilirubin in Na_2CO_3 is mixed to albumin (dissolved in PO_4 buffer 0 1 M pH 6 6) 0 5 ml of each mixture is layered on columns conditioned respectively at pH 7 0 and 7 4 Bilirubin from peaks 1 and 2 is measured and expressed as of the total bilirubin recovered

On the other hand it is possible to compensate for the acidosis effect by increasing the albumin concentration These in vitro experiments once more emphasize the importance of acidosis in kernicterus production and the protective effect of albumin

The number of bilirubin molecules which *can be bound by one albumin molecule* has been determined by several means with various results (9 10 16 21 25 27)

Furthermore using the same technique the albumin bilirubin molar ratio varies according to whether one is working with crystallized or purified albumin or with serum (10 22 25)

With Sephadex gel filtration at pH 7 3, we found that 78 mg of albumin is necessary to bind 1 mg of bilirubin

When albumin and bilirubin molecular weights are chosen as 69 000 and 585 respectively the calculation shows that in vitro 1 albumin molecule binds 1 5 bilirubin molecule in our experimental conditions This observa*tion is consistent with the results with Sepha*dex obtained by Keenan et al (10) With the same technique Keenan et al (10) and Kaufman et al (9) working on sera found that 1 albumin molecule binds 1 bilirubin molecule

Albumin purification steps may have modified the binding capacity of albumin although its electrophoretic and immunologic properties are identical to that of albumin in serum This commercial albumin is probably cleared out of

Acta Pædiat Scand 60 33-38 1971

SEPARATION BY GEL FILTRATION AND MICRODETERMINATION OF UNBOUND BILIRUBIN

II Study of Sera in Icteric Newborn Infants

P ZAMET and F CHUNGA

From the Centre de Recherches Biologiques Neonatales Paris France

Numerous studies *in vitro* and *in vivo* have established that bilirubin cytotoxicity involved in kernicterus from neonatal jaundice is related to the only ultrafilterable fraction not albumin bound (19 20) This unbound bilirubin can be extracted from cerebral tissues after kernicterus in man as well as in animals (3 4 7 29) it impairs the cellular functions (4) chiefly through a prevalent uncoupling effect blocking the oxidative phosphorylation (2 4 17 26 31)

The absence of toxicity of the protein bound bilirubin has been suggested by its exclusively extra-cellular distribution and is now well established (2 4 17 18) For these reasons separation of the free bilirubin and determination of its serum level has been of great interest Technical difficulties related to the low levels of unconjugated bilirubin that may be present in jaundice sera have been solved Separation can be precisely performed through gel filtration with Sephadex (1 6 9 10) Recently this method allowed small quantities of free bilirubin to be extracted and accurately measured (1)

The purpose of the present study was to demonstrate the possible presence of unbound bilirubin during neonatal jaundices to measure its levels and to study whether any correlation could be shown in relation to total bilirubinemia

MATERIAL AND METHODS

45 blood samples were obtained from 35 jaundiced newborns

23 samples were taken from infants with erythroblastosis fetalis (Rhesus or ABO incompatibility—Table I) and 22 from premature infants (Table 2) Most measurements were carried out on blood obtained by the first increment at the start of exchange transfusion In some cases a second sample was taken at the end of the exchange procedure

Two infants had symptoms of kernicterus 5 autopsies were performed

Repeated serum assessments of total bilirubin (Fig 2) free bilirubin free fatty acids total protein levels and of albumin were carried out after infusion of lipid emulsion (2 g/kg) and amino acids solution[1] (0 127 g nitrogen/kg) in a jaundiced infant This infant had a gestational age of 35 weeks and had multiple malformations potentially causing death but no respiratory distress and no cardiac failure (unfortunately no autopsy was performed)

Total bilirubin determinations were carried out by the Michaelsson's technique (16) Free bilirubin was separated by gel filtration with Sephadex then measured with the Chunga-Lardinois micromethod (1) Total serum protein content was determined by the biuret method after polyacrylamide electrophoresis of a serum sample the albumin fraction was evaluated on a spectrophotometer recorder Free fatty acids were assessed with the Laurell's micromethod (11)

RESULTS

General results

Among the 45 samples obtained just before exchange transfusion or in the course of jaundice 25 had noticeable amounts of free bili-

[1] Intralipide 10% and Aminosol 100 Vitrum

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Table 1 *Newborns with Rhesus or A B O incompatibility Pre and Post exchange transfusion (E T) samples*

Case	Sex	Term (weeks)	Birth weight (g)	Hours of life	Total Bilirubin mg/100 ml		Unbound Bilirubin mg/100 ml	
					Pre E T	Post E T	Pre E T	Post E T
(*a*) Newborns with Rhesus Incompatibility								
1 Yad	♂	36	1 940	4	9 5	2 4	0 04	0 00
2 Bru	♂	30	1 950	9	19 0	8 3	0 24	0 08
3 Lau	♀	38	3 080	70	28 0	5 3	0 36	0 20
4 Lou	♀	38	3 720	43	15 0	6 2	0 16	0 32
5 Ber	♂	37	3 330	57	18 0	8 2	0 00	0 00
6 Mez	♂	40	3 330	62	16 0		0 00	
7 Dum	♀	36	2 750	24	14 8		0 16	
8 Bou	♂	39	2 750	44	18 8		0 05	
9 Vei	♀		2 300	59	21 2		0 20	
10 Lon	♀	41	3 230	29	17 2		0 00	
11 Lon	♀	41	3 230	47	14 2		0 08	
12 Don	o	38	3 700	22	18 0		0 00	
13 Bra	♀	41	2 780	86	27 0		0 00	
14 Bra	♂	41	2 300	86	38 0		0 56	
15 Adr	o	41	3 000	99	35 2		1 00	
(*b*) Newborns with A B O Incompatibility								
1 Mat	♂	41	3 340	55	23 8	6 4	0 12	0 00
2 Bur	♂	38	3 660	79	23 0	6 3	0 16	0 00
3 Pla	♂	38	3 400	91	23 5	6 0	0 08	0 00
4 Tal	o	41	3 100	61	19 5	6 8	0 16	0 00
5 Dra	♂	41	3 250	69	22 2	8 1	0 16	0 00
6 Que	♀	41	3 960	60	27 6		0 12	
7 Que	♀	41	3 960	62	19 0		0 00	
8 Rac	♀	38	2 280	73	30 0		0 36	

rubin (0 1 mg/100 ml or more) Levels ranged from 0 1 mg to 1 mg/100 ml, but in only five cases (3 erythroblastosis and 2 prematures) the level exceeded 0 3 mg/100 ml Levels in excess of 0 5 mg/100 ml were exceptional in this series, only two cases were observed and both had erythroblastosis fetalis These infants were admitted too late at the special care unit and their total bilirubin levels were above 35 mg/100 ml

Among the 25 samples with noticeable free bilirubin levels 12 had a total bilirubin of 20 mg/100 ml or more, 8 infants (all prematures) had total bilirubin levels less than 18 mg/100 ml, one of the latter had a fairly high amount of free bilirubin (0 35 mg/100 ml) with a total bilirubin of 17 8 mg/100 ml another had a significant level of free bilirubin (0 21 mg/100 ml) with a total bilirubin of 12 mg/100 ml

Generally speaking there is no correlation between the amount of unbound bilirubin and the total bilirubin (Fig 1)

Except for one case, all the free bilirubin levels after exchange transfusion were much lower Often the unbound fraction had completely disappeared

Individual cases

(*a*) In three cases a kernicterus was observed Two infants had clinical symptoms, both were late admissions with extremely high levels of total bilirubin

In one case (sample number 14 Table 1) total bilirubin was up to 38 mg/100 ml and free bilirubin level was 0 56 mg/100 ml This infant had a twin whose total bilirubin was 27 mg/100 ml without any detectable free bilirubin the twin did not show symptoms of kernicterus (sample number 13, Table 1)

In the second case total bilirubin was 35 2 mg/100 ml and free bilirubin was 1 mg/100 ml

A case of kernicterus occurred in a premature infant of 28 weeks gestationnal age with

Table 2 *Icteric premature infants without incompatibility*

Case	Sex	Term (weeks)	Birth weight (g)	Hours (H) or days (D) of life	Total Bilirubin mg/100 ml Pre E.T	Total Bilirubin mg/100 ml Post E.T	Unbound Bilirubin mg/100 ml Pre E.T	Unbound Bilirubin mg/100 ml Post E.T
a) Pre and post Exchange Transfusion (ET) samples								
1 Poi	♂	43	2 450	H 63½	21 5	4 7	0 20	0 00
2 Pig	o	33	1 940	D 6	20 3	2 6	0 12	0 00
3 Zer	♂	36	2 400	D 4½	16 4		0 16	0 04
4 Leb	♀	34	*1 600*	D 4	20 0	8 9	0 16	0 00
5 Ody	♀	32	1 500	H 72	20 2	6 6	0 24	0 06
6 Ody	♀	32	1 500	H 81	19 1	7 8	0 22	0 00
7 Fic	♂	36	3 200	D 5½	17 2	5 2	0 00	0 00
(*b*) *Sample during the course of hyperbilirubinemia*								
8 Fic	o	36	3 200	D 6	11 4		0 00	
9 Leb	♀	34	1 600	D 4	20 0		0 05	
10 Cas	♂	37	2 800	H 82	12.0		0 21	
11 Rey	♂	28	1 000	D 5½	15 4		0 16	
12 Rey	o	28	1 000	D 6	15 2		0 16	
13 Ang	♂	30	2 210	D 5	15 8		0 00	
14 Pig	♂	33	1 940	D 6½	16 6		0 00	
15 Zer	♂	36	2 580	D 5½	18 2		0 00	
16 Bai	×		1 270	H 74	15 5		0 10	
17 Pat	♂	37	2 650	D 4½	17 8		0 35	
18 Pat	♂	37	2 650	D 5	17 4		0 16	
19 Lep	×	36	2 370	D 4	18 6		0 00	
0 Lep		36	2 370	D 6	17 6		0 00	
21 Lep	×	36	2 370	D 6½	17 9		0 00	
22 Deb		34	1 700	D 3½	14 2		0 10	

a birth weight of 1 000 g two samples showed an unbound bilirubin level of 0 16 mg/100 ml while total bilirubin was *15 4* and *15 2 mg/* 100 ml respectively This infant had no respiratory distress acid base balance was correct except in the last six hours before death (pH 7 03 Pa_{CO_2} 56 mmHg B E −17 mEq Pa_{O_2} 57 mmHg) At the autopsy a small hemorrhage was noted in the germinative zone Kernicterus was evident with yellow *pigments in habenula* in the third cranial nerve nucleus and in the nuclei situated under the floor of the fourth ventricle

(*b*) Four additional autopsies were performed on infants who had presented free bilirubin levels ranging from 0 1 to 0 24 mg/100 ml None of them showed any sign of kernicterus Three were prematures (birth weight 1 500 g, 1 270 g 1 950 g) and they had an intraventricular hemorrhage

(*c*) The consequence of infusing a lipid emulsion (essentially free fatty acids) and amino acids solution is presented in Fig 2

At the beginning of the infusion total bilirubin was 14 2 mg/100 ml and only an in*significant amount of unconjugated bilirubin* could be detected (0 08/100 ml) Half an hour after the infusion an important rise of the free bilirubin level was observed (0 44 mg/100 ml) followed by a slow decrease The initial level was reached after 96 hours During this period an isolated infusion of amino acids did not *modify the decrease rate*

When the total bilirubin was 5 7 mg/100 ml with no free bilirubin infusion of lipids and amino acids did not trigger any appearance of free bilirubin

COMMENTS

The most striking finding is that no correlation can be established between the total bilirubin level and the amount of unbound bilirubin (Fig 1) neither in infants with erythroblastosis fetales nor in prematures (Tables 1 and 2)

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Case	Sex	Term (weeks)	Birth weight (g)	Hours of life	Total Bilirubin mg/100 ml Pre E T	Total Bilirubin mg/100 ml Post E T	Unbound Bilirubin mg/100 ml Pre E T	Unbound Bilirubin mg/100 ml Post E T
(*a*) Newborns with Rhesus Incompatibility								
1 Yad	♂	36	1 940	4	9 5	2 4	0 04	0 00
2 Bru	♂	30	1 950	9	19 0	8 3	0 24	0 08
3 Lau	♀	38	3 080	70	28 0	5 3	0 36	0 20
4 Lou	♀	38	3 720	43	15 0	6 2	0 16	0 32
5 Ber	o	37	3 330	57	18 0	8 2	0 00	0 00
6 Mez	♂	40	3 330	62	16 0		0 00	
7 *Dum*	♀	*36*	*2 750*	*24*	*14 8*		*0 16*	
8 Bou	o	39	2 750	44	18 8		0 05	
9 Vei	♀		2 300	59	21 2		0 20	
10 Lon	♀	41	3 230	29	17 2		0 00	
11 Lon	♀	41	3 230	47	14 2		0 08	
12 Don	♂	38	3 700	22	18 0		0 00	
13 Bra	♀	41	2 780	86	27 0		0 00	
14 Bra	♂	41	2 300	86	38 0		0 56	
15 Adr	♂	41	3 000	99	35 2		1 00	
(*b*) Newborns with A B 0 Incompatibility								
1 Mat	♂	41	3 340	55	23 8	6 4	0 12	0 00
2 Bur	♂	38	3 660	79	23 0	6 3	0 16	0 00
3 Pih	♂	38	3 400	91	23 5	6 0	0 08	0 00
4 Tal	o	41	3 100	61	19 5	6 8	0 16	0 00
5 Dra	♂	41	3 250	69	22 2	8 1	0 16	0 00
6 Que	♀	41	3 960	60	27 6		0 12	
7 Que	♀	41	3 960	62	19 0		0 00	
8 Rac	♀	38	2 280	73	30 0		0 36	

rubin (0 1 mg/100 ml or more) Levels ranged from 0 1 mg to 1 mg/100 ml, but in only five cases (3 erythroblastosis and 2 prematures) the level exceeded 0 3 mg/100 ml Levels in excess of 0 5 mg/100 ml were exceptional in this series, only two cases were observed and both had erythroblastosis fetalis These infants were admitted too late at the special care unit and their total bilirubin levels were above 35 mg/100 ml

Among the 25 samples with noticeable free bilirubin levels, 12 had a total bilirubin of 20 mg/100 ml or more, 8 infants (all prematures) had total bilirubin levels less than 18 mg/100 ml, one of the latter had a fairly high amount of free bilirubin (0 35 mg/100 ml) with a total bilirubin of 17 8 mg/100 ml another had a significant level of free bilirubin (0 21 mg/100 ml) with a total bilirubin of 12 mg/100 ml

Generally speaking there is no correlation between the amount of unbound bilirubin and the total bilirubin (Fig 1)

Except for one case all the free bilirubin levels after exchange transfusion were much lower Often the unbound fraction had completely disappeared

Individual cases

(*a*) In three cases a kernicterus was observed Two infants had clinical symptoms, both were late admissions with extremely high levels of total bilirubin

In one case (sample number 14 Table 1) total bilirubin was up to 38 mg/100 ml and free bilirubin level was 0 56 mg/100 ml This infant had a twin whose total bilirubin was 27 mg/100 ml without any detectable free bilirubin the twin did not show symptoms of kernicterus (sample number 13, Table 1)

In the second case total bilirubin was 35 2 mg/100 ml and free bilirubin was 1 mg/100 ml

A case of kernicterus occurred in a premature infant of 28 weeks gestationnal age with

other hand the presence of unbound bilirubin even in fairly high levels does not necessarily result in lesions of central gray nuclei (samples 5 6 10 16 Table 2 2 Table 1) Finally when there is no free bilirubin there will be no risk of kernicterus This is suggested by experimental studies (2 4 17 18) and the case history of the twins in which one had kernic terus with high total bilirubin and high free bilirubin while the other free of symptoms was in spite of a high level of total bilirubin to g ther wi h an insignificant amount of free bili rubin

These data point out that the presence of unbound bilirubin only represents an index of risk other factors certainly play a role in pro ducing cerebral lesions at a given level of free bilirubin The status of the blood brain barrier (or perhaps the permeability of cell membrane by itself) may undergo certain changes through anoxia or hypercapnia (12)

It is usually accepted that *gestational age* plays a part in the degree of permeability of the blood brain barrier It should however be noticed that some authors argue against the immaturity of the blood brain barrier in any newborn during the *first days of life (4 27)* The individual varying sensitivity and chiefly the duration of exposure of brain tissues to unbound bilirubin (in relation to the *prompti tude* of exchange transfusion) are likely to in flu nce the development of kernicterus

Larger series are of course necessary to establish correlations between the *free bilirubin level* (with regard to the exact duration of this plasmatic level) and the occurrence of kernic terus as assessed by pathology or by *careful* clinical evaluation after a long follow up

SUMMARY

Extraction of unbound bilirubin by Sephadex gel filtration and measurement by a micro-method were carried out in 45 sera from jaun diced newborns (hemolytic disease or prema tures) In about half the cases some free bili rubin with values in the range 0 1 to 1 mg/100 ml was detected Levels above 0.5 mg/100 ml were exceptional (only 2 cases) Five autopsies were performed in prematures with unbound bilirubin levels above 0 1 mg/100 ml and one kernicteru was found Two infants with un bound bilirubin levels of more than 0 5 mg/ 100 ml had clinical symptoms of kernicterus No symptoms of kernicterus were observed in infants with high total bilirubin together with an *insignificant amount* of unbound bilirubin

The main interest of this method lies in the direct assessment of a toxic element originat ing from varying bilirubin and albumin levels and the micro-environment of the bilirubin al-bumin *binding*

But other factors such as the cell mem brane permeability and/or the duration of ex posure are likely to play a *prominent role*

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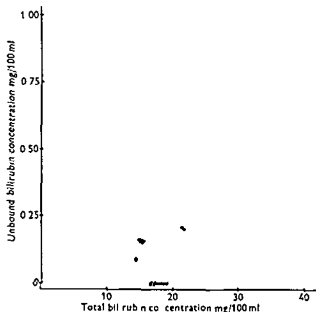


Fig 1 Relationship between total and unbound bili rubin

This absence of correlation might be ex pected as long as the bilirubin albumin bind ing is known to depend upon the levels of bili rubin and albumin, and on the environmental conditions of acidbase balance (12 13 21) It depends also on other factors such as serum lipoproteins (30) and substances competing with bilirubin at the albumin site, for example bile salts (5) free fatty acids (7 22, 24) hema- tin (20) and various anionic exogenous com- pounds (7, 8 14 15, 20 23)

The competitive action of free fatty acids is shown by the almost experimental case history on Fig 2 Consequently, the intra venous infusion of lipid emulsions is a quite dangerous method in jaundiced newborn it should not be employed unless the level of the total bilirubin is low

The absence of relation between total bili rubin and unbound bilirubin can be compared with the results of Odell (23) This author found no correlation between the saturation of the serum albumin and the concentration ratio of the bilirubin to total protein of the serum in infants with erythroblastosis fetalis How ever, a linear correlation was found between these two parameters in non hemolytic jaund ices

All techniques dealing with the assessment of residual capacity of fixation for bilirubin by serum proteins (P S P , HBABA, or salicylate (15, 23, 25, 29)) have the drawback of being indirect methods

The advantage of free bilirubin measure ment is that the amount of this toxic fraction results from a balance between bilirubin and albumin in a variable microenvironment

The small number of autopsies (5) and kernicterus (2) in this series is inadequate to prove any correlation between the presence of free bilirubin and kernicterus However these preliminary data suggest that kernicterus can be observed in spite of low levels of total bili rubin (samples 11 and 12, Table 2) On the

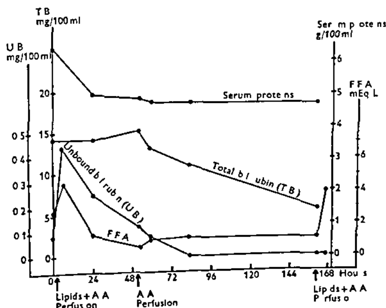


Fig 2 Variations of total and unbound bilirubin F F A total proteins under *lipids and amino acids perfusion* (al bumin accounts for 50–53 of total proteins)

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A FOLLOW UP STUDY OF PHYSICAL GROWTH FOLLOWING FAILURF TO THRIVE WITH SPECIAL REFERENCE TO A CRITICAL PERIOD IN THE FIRST YEAR OF LIFE

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It has been suggested that if a child suffered from some condition in the first year which slowed his normal rapid growth the child might not achieve his full growth potential after correction of that condition (14) There has been a dearth of studies on the subsequent physical growth of children who had failure to thrive associated with major surgical intervention in the first year of life Similarly there has b en a lack of documented studies about the ex istenc of a postnatal critical period for physical growth caused by medical or surgical illnesses during the very early part of the first year

Poor nutrition during the suckling period in the rat followed by realimentation reduced the eventual size of the adult rat (16)

It was reported (3 9 29) that children who had failure to thrive had subsequent normal physical growth while others (1 2 7) found that more than one third of the children had subsequent growth failure The retarding ef fect of early malnutrition and undernutrition on subsequent physical growth has been re ported (8 22) The subsequent weight of children who had undergone neonatal surgical procedures was reported to be within normal limits (5 9) or hi hter than normal (6)

CLINICAL MATERIAL AND METHODS

A Failure to thrive samples

Children were included in the study if their weight during the first year fell below the 3rd percentile using Tanner s charts A low birth child (birth weight below 2 500 g) was included if his weight fell progressively below the 3rd percentile

Children were chosen after reviewing the index cards medical records and theatre books as far back as 1959 under the following diagnoses idiopathic failure to thrive feeding problems steatorrhoea coeliac disease fibrocystic disease of the pancreas idiopathic hypercalcaemia disaccharidase deficiency renal acidosis nephrogenic diabetes insipidus congenital heart disease mental retardation (except mongols and cerebral palsy) oesophageal atresia Hirschprung s disease intestinal ob truction duodenal atresia exomphalos and patent ductus arteriosus (PDA) ligated in and after the first year (up to 8 years)

151 children were considered to have had failure to thrive Beside these another 14 cases were chosen whose PDA was ligated after the first year and who were below the 10th percentile in weight before the operation They were traced by a letter requesting the parents to bring the child to the Children s Hospital Sheffield The consultant paediatricians were requested to examine children who were living far away Out of 165 cases data on 132 patients were obtained i e 74 response and of these 25 patients were examined by consultant paediatricians These 132 cases were placed in three groups

Treated group includes 77 cases in whom the cause of failure to thrive was corrected completely with the exception of PDA cases ligated after the first year

Untrea ed group includes 35 cases in whom the cause was or could not be corrected

PDA group ligated after the 1st year includes 10 cases

B Control samples

Control group includes 75 healthy children from the fracture clinic

Sibl ng group includes 24 children 12 were siblings of children in the treated group 8 were siblings of children in the untreated group and 4 were siblings

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Key words Unbound bilirubin determination risk of kernicterus newborns

children aged 5 years and over For children below 5 years Hammond s (11) tables were used

Head circumference Standard scores were calculated from Westropp & Barber (?8) tables for children aged up to 8 years and Watson & Lowrey (27) tables for children older than this

Triceps and Subscapular subcutaneous folds Percentiles were obtained by plotting the measurements on Tanner & Whitehouse (24) charts

Chest circumference The Harvard and Iowa percentile tables published in Nelson Textbook of Pediatrics (1964) were used to obtain percentile groups

Skeletal age The standards of the Greulich & Pyle Atlas (10) as well as Stuart s tables published in this Atlas were used to calculate the standard scores of skeletal age

To homogenize the differences due to age and sex the measurements were converted into percentile groups and/or standard scores The standard scores were calculated using the following equation standard score $= X - \bar{X}/S\,D$ where X is th measurement $\bar{X}$ is the mean for th age and sex and S D is the standard deviation of the mean

The decimal age was used throughout (25)

RESULTS

A *Using the percentile distribution*

In Table 2 the total number and percentage of children who were below the 3rd percentile in height and in weight in each group and diagnostic category were shown

The percentile distribution of the control group for height and for weight did not differ significantly from that of the normal population as represented by Tanner's distribution (using chi square test) In the treated group 16 lay below the 3rd percentile in both height and weight in the surgically treated cases

Table 3 *Triceps subcutaneous fold percentile distribution*

Percentile groups		<10	10–50	>50
Control	n		11	61
(n = 72)	%		15	85
Treated[a]	n	10	22	27
(n = 59)	%	17	37	46
Untreated[b]	n	7	11	12
(n = 30)	%	23	37	40

[a] X^2_2 (control and treated) = 25 68 $p < 0\,001$
[b] X^2_2 (control and untreated) = 24 70 $p < 0\,001$

Table 4 *Subcapsular subcutaneous fold percentile distribution*

Percentile groups		<10	10–50	>50
Control	n	12	31	29
(n = 72)	%	17	43	40
Treated	n	16	29	13
(n = 59)		28	50	22
Untreated[b]	n	8	16	6
(n = 30)	%	27	53	20

[a] X^2_2 (control and treated) = 4 45 $p < 0\,20$
[b] X^2_2 (control and untreated) = 4 73 $p < 0\,10$

Table 5 *Chest circumference percentile distribution*

Percentile group		<10	10–50	>50
Control	n	6	15	54
(n = 75)	%	8	20	72
Treated	n	14	20	29
(n = 58)		24	36	41
Untreated[b]	n	11	7	11
(n = 35)	%	38	24	38

X^2_2 (control and treated) = 13 12 $p < 0\,01$
[b] X^2_2 (control and untreated) = 15 25 $p < 0\,001$

26 % in height and 22 % in weight were below the 3rd percentile while in the medically treated cases 21 % in height and 42 in weight were below the 3rd percentile In the untreated group 37 lay below the 3rd percentile in both height and weight

In the treated group of the 19 children who were below the 3rd percentile in height at follow up 8 cases had completed their surgical treatment in the first year 3 cases of oesophageal atresia had primary anastomosis early in the first year followed by serial oesophageal dilation 3 cases of oesophageal atresia had colonic replacement in the second and third year one case of Hirschsprung s disease had the second stage in the second year and the rest were medical cases

The triceps subcutaneous fold percentile distribution (Table 3) and not the subscapular one (Table 4) was significantly thinner in

Table 1 *Pertinent data concerning the number age range, mean age and sex in the different groups*

	Treated	Untreated	Sibling	Control	PDA ligated after 1st year
Number	77	35	24	75	10
Age range	0 81–10 53	0 87–11 55	1 10–9 13	0 92–11 01	2 40–9 49
Mean age	4 51	4 70	5 48	5 89	5 84
Males	44	18	11	37	6
Females	33	17	13	38	4

of children whose patent ductus arteriosus was ligated after the first year

Table 1 summarizes the pertinent data concerning different groups

Methods of measurement

The standing height was measured by a Harpenden Portable Stadiometer Gentle upward traction was applied to the mastoid processes The head was positioned so that the child was looking directly forward with the Frankfort plane (lower border of the orbit to the external auditory meatus) and the bi auricular plane horizontal

The supine length was measured by a Harpenden supine length table

The weight was measured by an Avery weight chair which was accurate to the nearest 50 g The child was weighed either nude or wearing his under pants only (no correction was made for these)

The head circumference was measured by a cloth tape measure applied around the head touching the glabella anteriorly and the posterior occipital protuberance posteriorly The measure was checked against stretching

The chest circumference was measured by a cloth tape measure applied around the chest at the level of the xiphi sternal joint midway between inspiration and expiration

The triceps and the subscapular subcutaneous folds were measured by a Harpenden skin caliper according to Tanner & Whitehouse technique (24)

The skeletal age was assessed personally from an X ray of the left hand and wrist using the Greulich & Pyle Atlas (10) Some of the X rays were assessed by the local radiologists using the same atlas

Standards used Height and Weight The percentile groups were obtained from Tanner s charts The standard scores for height were calculated from the tables of Tanner et al (25) The standard scores for weight were calculated from Scott s L C C (21) tables for

Table 2 *Total number and percentage of children below the 3rd percentile in height and weight in different groups and diagnostic sub groups*

Group	Total no	Height *n*		Weight *n*	
A *Control*	75			1	1 3
B *Sibling*	24			1	12 5
C *Treated*	77	19	24 0	21	28 0
Oesophageal atresia	16	6	37 5	6	37 5
Hirschsprung s disease	9	1	11 0	2	22 0
Intestinal obstruction	15	4	26 7	2	13 3
Duodenal atresia	7	2	28 6	1	14 3
Exomphalos	4	1	25 0		
PDA (ligated in 1st year)	7	1	14 5	2	28 6
Failure to thrive	14	3	21 4	7	50 0
Idiopathic hypercalcaemia	4	1	25 0	1	25 0
Nephrogenic diabetes insipidus	1				
D *Untreated*	35	17	48 6	19	54 2
Oesophageal atresia (1st stage)	2	1	50 0	1	50 0
Hirschsprung s disease + ileostomy	1	1	100 0		
Fallot s tetralogy	4	2	50 0	2	50 0
Acyanotic congenital heart disease	12	3	25 0	6	50 0
Mental deficiency	13	10	77 0	10	77 0
Fibrocystic disease of pancreas	3				
E *PDA (ligated after 1st year)*	10	1	10 0	2	20 0

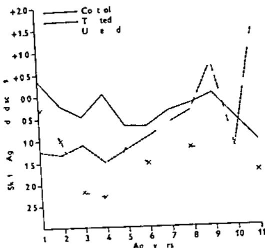


Fig 2 Age trends in mean skeletal age standard scores

ard scores) than males (−0 73±1 04 standard scores) (t=12 10 p<0 001)

There was only slight differences in the post operative growth picture between cases of PDA ligated in and after the first year

Effect of birth weight and gestational age on subsequent height

The mean birth weight of a control sample consisting of 223 children born in the Jessop Hospital in 1961 was 3 202±0 638 kg The mean birth weight of the treated group was 2 834±0 603 kg and it was significantly lower than that of the control birth weight (p<0 01) The correlation between birth weights and heights was r+0 119 (not significant) The correlation between gestational ages and heights was r+0 039 In the untreated group the mean birth weight was 2 926±0 533 kg and was significantly lower than that of the control (p<0 01) In the treated group 23° of the children had low birth weight (below 2 500 g) and half of them were small for dates, while the incidence of low birth weight in the untreated group was 17°₀ and all of them were small for dates

Effect of mother s height on subsequent height of their children

Most of the mothers were measured personally

Treated group The mean of the mother s height (160±7 0 cm) was not significantly lower than that of the control group (162 5±5 1 cm) The correlation between the heights of mothers and heights of their children was r+0 344 (p<0 01) Mothers of children who were below the 3rd percentile had a slightly shorter mean height than mothers of children over the 3rd percentile

Untreated group The mean of mother s height (160 8±6 0 cm) was not significantly lower than that of the control group The correlation between the heights of the mothers and the heights of their children was r+0 006

Effect of father s height on subsequent height of their children

Most of the data were obtained from the mothers

Treated group The mean of the father s

Table 8 *Effect of age of onset of failure to thrive on physical growth in the treated group*

Number between brackets denotes the number of children examined

Age of onset of failure to thrive	n	Height Standard scores		Weight Standard scores		Head circumference Standard scores	
		Mean	S D	Mean	S D	Mean	S D
Onset in 1st two months	57	−1 07	±1 51	−0 93	±1 07	−0 98 (54)	±1 05
Onset in 3rd and 4th months	10	−1 02	±1.25	−1 09	±1 03	−1 06	±0 85
Onset in 5th to 1 th months	10	−1 15	±0 98	−1 54	±1 40	−1 00	±0 90

Table 6 *Means, standard deviations of control, treated untreated and sibling groups on follow up*
The significance level compared with control group is marked by asterisks *** $p<0.001$ ** $p<0.01$ * $p<0.05$

Group	Height Standard scores Mean (*n*)	S D	Weight Standard scores Mean (*n*)	S D	Head circumference Standard scores Mean (*n*)	S D	Skeletal age Standard scores Mean (*n*)	S D
Control	+0 25 (75)	±0 79	+0 04 (73)	±0 91	+0 02 (75)	±0 98	−0 37 (70)	±1 18
Treated	−1 09*** (77)	±1 41	−1 03*** (76)	±1 05	−0 98*** (76)	±1 06	−0 98** (75)	±1 57
Untreated	−1 62*** (35)	±1 39	−1 86*** (35)	±0 93	−1 12*** (35)	±1 15	−1 67*** (35)	±1 39
Sibling	−0 14 (24)	±1 06	−0 42* (24)	±1 09	−0 55*** (24)	±1 04	−0 74 (23)	±1 68

treated and untreated groups when compared with that of the control group

The chest circumference percentile distribution (Table 5) was significantly smaller in the treated and untreated groups than that of the control group

B *Using the standard scores*

From Table 6, we observe that the means for weight, and head circumference of the sibling group were significantly lower than those of the control group The means for height weight, head circumference and skeletal age of the treated and untreated group were significantly lower than those of the control group However only the means for height and weight of the treated group were significantly lower than those of the siblings

Since the sibling group consisted of siblings of the three main groups, height and weight of the ill and healthy siblings of the treated group were compared From Table 7 bigger differences were observed although they were not statistically significant due to the small size of the samples

In the treated group, the mean height at each year (from 3 to 8 years) was significantly lower than the corresponding mean of the control group (Fig 1) The mean skeletal age of the treated group at each year was slightly lower than the corresponding mean of the control group (Fig 2)

The only significant sex difference in the treated group was that females had a smaller mean head circumference (− 1 30 ± 1 01 stand

Table 7 *Comparison between the ill and healthy sibling of the treated group*

Groups	*n*	Height standard scores Mean	S D	Weight standard scores Mean	S D
Ill cases	12	−1 47	±2 28	−1 34	±1 56
Healthy siblings	12	−0 07	±1 00	−0 46	±0 93
Significance		$t=1.86$	$p<0.1$	$t=1.50$	$p<0.2$

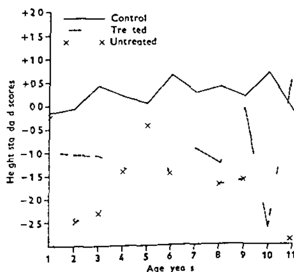


Fig 1 Age trends in mean height standard scores

Table 9 *Effect of duration of failure to thrive on physical growth in the treated group*

Duration of failure to thrive	n	Height standard scores		Weight standard scores		Head circumference standard scores	
		Mean	S D	Mean	S D	Mean	S D
Duration ≤4 month	12	−0 76	±1 53	−0 71	±1 01	−0 98	±1 25
Duration >4–8 months	10	−1 70	±1 34	−0 89	±1 41	−0 57	±0 98
Duration >8 months	18	−1 47	±1 34	−1 41	±0 94	−1 49	±0 86

tion increased in proportion to the duration of failure to thrive and a significant difference was found between the mean head circumference of the longest and moderate duration sub groups ($p<0\ 001$) The incidence of low birth weight in the two shorter periods combined was 27% compared to 22 in the longest dura tion group

Effect of age of correction of failure to thrive on subsequent physical growth in the treated group

Age of correction means the age at which the weight rose above the 3rd percentile in weight in the first year From Table 10 we observe that there was minimal difference in the subsequent physical growth between early and late correction However if the child s failure to thrive was not corrected during the first year there was more significant retardation in the three parameters tested ($p<0\ 001$) than if the child s failure to thrive was corrected at any time in the 1st year

The relation of age of correction of failure to thrive to the other variables is shown in Table 11 We observe that there was a high incidence of prolonged periods of failure to thrive in the uncorrected group Incidence of early onset of failure to thrive was similar in corrected and uncorrected groups The older mean age of the uncorrected group might curtail their chance of catch up

Effect of number of percentile channels lost during failure to thrive on subsequent physical growth in the treated group

Children with low birth weight were excluded The number of percentile channels lost is an indication of failure to thrive However losing 4–5 channels caused less retardation in the parameters tested than losing 1–2 channels

DISCUSSION

It is difficult to exclude the effect of illness from that of genetic endowment The ideal con trol group would consist of children of this group who were also below the 3rd percentile in weight in their first year but this would be practically impossible to collect

The significantly retarded growth of the sur gically treated cases did not conform to the within normal weight reported by Eckstein (5) The non significant differences between

Table 10 *Effect of age of correction of failure to thrive on physical growth in the treated group*

Age of correction of failure to thrive	n	Height standard scores		Weight standard scores		Head circumference standard scores	
		Mean	S D	Mean	S D	Mean	S D
≤4th month	12	−0 76	±1.54	−0 71	±1 01	−0 93	±1.25
4th–1 th month	7	−0.55	±1 49	−0.22	±0 60	−0 13	±0 83
Corrected in 1st year	19	−0 61	±1 48	−0 53	±0 89	−0 63	±1 15
Not corrected in 1st year	21	−1 72	±1 06	−1 65	±0 95	−1 54	±0 73

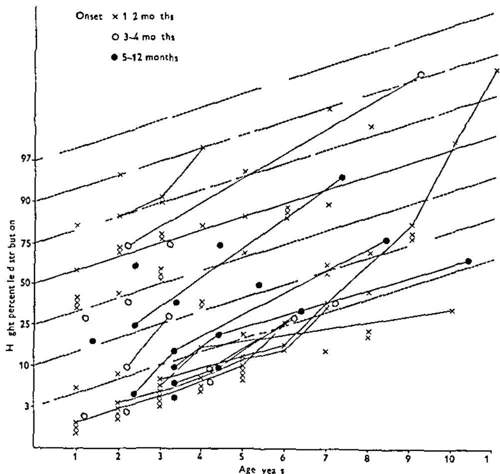


Fig 3 Effect of age of onset of failure to thrive on subsequent height percentiles in relation to chronological age Lines joining the marks concern children at successive follow ups

height (171 5 ± 8 9 cm) was significantly lower than that of the control group (174 8 ± 8 0 cm) ($p < 0\ 01$) The correlation between the heights of the fathers and the heights of their children was $r + 0\ 300$ ($p < 0\ 02$) The mean of father's height of children below the 3rd percentile was similar to that of fathers of children above the 3rd percentile

Untreated group The mean of father s height (171 3 ± 7 5 cm) was significantly lower than that of the control group ($p < 0\ 05$) The correlation between the heights of fathers and the heights of their children was $r - 0\ 799$

Effect of age of onset of failure to thrive on subsequent physical growth in the treated group

Age of onset means the time at which the child s weight fell below the 3rd percentile in the first year We observe from Table 8 that the very early onset of failure to thrive did not lead on the average to more retarded growth than later onset The ensure that the heterogenicity of the diagnoses did not affect the results, means of height standard scores of cases of idiopathic failure to thrive with early (5 cases) and late (9 cases) onsets were calculated Those with late onset were on the average slightly shorter than those with early onset From Fig 3, we observe that with different times of onset there was no significant difference in the height percentiles at the final follow up at different ages With onset in the first two months 14 cases were on or above the 50th percentile (24 6 %) while 15 cases were below the 3rd percentile (26 %) with a later onset 2 cases were on or above the 50th percentile (10 %), while 4 cases were below the 3rd percentile (20 %)

Effect of duration of failure to thrive on subsequent physical growth in the treated group

The duration here means the time spent below the 3rd percentile in weight in the first year From Table 9 we observe that growth retarda

In the present treated group 19 cases were below the 3rd percentile

Since illness associated with failure to thrive especially when prolonged and at any time in the first year caused significant growth retardation every effort should be made to tide the child over that period to hasten his recovery and to shorten this period In the surgically treated children this requires particular attention to early and late post-operative nutrition and adequate fluid and electrolyte balance

SUMMARY

This was a follow up study of physical growth of children who have had failure to thrive It comprised 77 children in whom the cause was corrected and 35 children with persistent organic cause together with 10 children in whom the patent ductus arteriosus was ligated after the first year The treated group consisted of surgical and medical cases Their pattern of growth was compared with that of a control group composed of 75 children from the fracture clinic and 24 siblings all from the same age range and nearly the same mean age Seven parameters of growth were tested Significant retardation in six parameters was noted in both the treated group (in which the cause of failure to thrive was removed) and the untreated group (in which the treatment of the cause was impossible) In the treated group the mean skeletal age was less retarded than the mean height while the mean head circumference of females was significantly more retarded than that of males There was only slight difference between early and late ligation of patent ductus arteriosus in subsequent physical growth Neither the very early onset of failure nor its severity caused a severe retardation within the treat d group Prolonged duration of failure to thrive or failure to correct the cause in the first year led to significant subsequent growth retardation within the treated group Parental height and birth weight played a less well defined role while socio-economic environment played a minor role in causing growth retardation in the treated group The first year can only be regarded as a relatively critical period concerning subsequent physical growth due to difficulty in separating the effect of illness from that of genetic endowment Growth retardation may represent another congenital abnormality independent of the original anomaly which was corrected The importance of reduced cell number was discussed

ACKNOWLEDGEMENTS

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Table 11 *Comparison between corrected and uncorrected cases of failure to thrive in relation to other variables*

	Corrected in 1st year	Not corrected in 1st year
Number	19	21
Mean age	4 years	6 years
Cases with low birth weight (below 2 500 g)	5	5
Cases with early onset of failure to thrive	13	14
Cases with duration of failure to thrive more than 8 months	1	17
Medical cases	6	9
Surgical cases	13	12

early and late ligation of PDA agree with other reports (17 26)

The non significant retardation of skeletal age in comparison with the significantly shorter height was also reported (12) in children who had minor illnesses, although they were only able to follow their cases up to 4 years of age This suggests that these children might end up as short adults unless the catch up in height exceeds that of skeletal age to overcome the early closure of the epiphysis Prader et al (20) stated that the catch up in height is stronger than the catch up in skeletal age in these young children

The significantly smaller mean head circumference of the females than that of the males in the treated group is in contrast with Tanner's (23) statement that females stand illness better than males

The very low or even negative correlation of parental heights with the heights of children of the untreated group suggests that such genetic effect was inhibited by the persistence of the organic cause

The significantly retarded growth of the sibling group suggests that its children came from a lower socio-economic environment than that of the control group The significantly retarded growth of the treated and untreated groups compared to that of the sibling group suggests that such lower socio economic environment was only partially responsible for their growth retardation

CONCLUSION

Neither the very early onset of illness associated with failure to thrive nor its severity caused a greater degree of growth retardation within the treated group Illness associated with prolonged duration of failure to thrive was the most major factor in producing significant growth retardation while the parental height birth weight and socio economic environment played a less well defined role In spite of the fact that onset of failure to thrive at any time in the first year caused significant growth retardation compared to the growth of the control group the first year can only be regarded as a relatively critical period since we cannot separate the effect of illness from that of genetic endowment

Children with congenital heart disease fail to grow after the corrective cardiac surgery These children have reduced cell number when this parameter was equated with the chronological age or height (4) Cell multiplication takes time, and too much time has been lost in these children—time when growth by multiplication was important

Since congenital anomalies, which were corrected by surgery were the principal cause of failure to thrive in the treated group, it may be that the smaller size of these children was another congenital anomaly not dependent on the original anomaly

The mean maximum serum growth hormone response in cases of dwarfism other than that caused by hypopituitarism was not significantly different from that response observed in a control group (15) while raised serum growth hormone levels were reported in cases of kwashiorkor (18) However serum growth hormone deficiency was reported in cases of dwarfism associated with emotional deprivation (19) The diagnosis of hypopituitarism is considered if the height was much below the 3rd percentile and lagged progressively below it (13)

In the present treated group 19 cases were below the 3rd percentile

Since illness associated with failure to thrive especially when prolonged and at any time in the first year caused significant growth retardation, every effort should be made to tide the child over that period to hasten his recovery and to shorten this period In the surgically treated children this requires particular attention to early and late post operative nutrition and adequate fluid and electrolyte balance

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INTENSIVE CARE OF SMALL PREMATURE INFANTS

II Postmortem Findings

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We have described previously the clinical features in 49 premature infants weighing less than 1 250 g of whom 32 were treated with intermittent positive pressure respiration (IPPR) (31) This report concerns the clinical pathological correlation in the 27 infants who died such a specific study has not been reported before

MATERIAL AND METHODS

Detailed postmortem examinations of 27 premature infants weighing less than 1 250 g who had been treated with IPPR were done between 16 and 38 hours after death the clinical details of these cases have been described elsewhere (31) Specifically tissue blocks from the hilus to the pleural surface were taken from every pulmonary lobe All tissue was fixed in neutral formalin and processed by standard paraffin techniques Hematoxylin and eosin or van Gieson stain were used Bacteriological specimens were taken from the posterior lobes of each lung. The following microscopic pulmonary abnormalities were noted the extent thickness and stage of resolution of hyaline membrane and the presence of haemorrhage inflammatory cell infiltration and fibrinous material in various parts of the lung It was sometimes difficult to distinguish hyaline membranes from fibrinous material but the basophilic staining of the latter and its involvement of bronchi were considered important points The presence of focal parenchymal necroses and intra and interalveolar fibrosis was also recorded These changes were graded as follows The hyaline membranes and haemorrhages were graded on the approximate extent of histological involvement as + (<10%) to + + + (>50%) The thickness of the membranes and the extent of their resolution as well as the density of the inflammatory cell infiltrate fibrosis and focal necroses was graded from slight (+) to severe (+ + +)

Supported in part by Grants from the Foundation for Paediatric Research in Finland

RESULTS

The clinical definitions symptomatology the diagnoses and the chest roentgenograms have been given previously (31)

Response to treatment

The material was divided into three categories *severe respiratory distress* (RD) *moderate RD* and *slight or no RD* (31)

The diagnoses duration of IPPR and survival times are shown in Table 1 Of the 11 infants with *severe RD* 5 required immediate postnatal intubation and assisted ventilation and the other 6 required this within 1–14 hours (mean 6 hours) Ten died within 72 hours with little or no response to treatment as measured by arterial pO and the clinical state One died after 3 months having developed the clinical and roentgenological signs of bronchopulmonary dysplasia (BPD) (18)

A much better response to IPPR was found in those with *moderate RD* IPPR treatment was needed less urgently (within 3–125 hours mean 44 hours) and the survival was longer In the last category IPPR was required even later (6–96 hours mean 57 hours) in 7 cases as a result of sudden unexpected apnoea The

Table 1 *Clinical radiological and autopsy findings, duration of IPPR and age at death in the 3 categories of RD*

RDS = respiratory distress syndrome HM = hyaline membranes PH = pulmonary hemorrhage IVH = intraventricular brain hemorrhage INF = infection BPD = bronchopulmonary dysplasia

Case no	Diagnosis Clinical	Diagnosis Autopsy	Chest X ray	Duration of IPPR (hours)	Age at death
Severe RD					
23	RDS PH	HM (PH)	II	86	3 days
24	RDS	HM	II	17	17 days
25	RDS PH	HM	III	14	14 days
26	INF IVH	INF (IVH)	—	15	15 days
27	IVH	IVH	I	23	23 days
28	RDS	HM	III	7	13 days
29	RDS	HM (IVH)	III	22	1 day 2 hours
30	RDS	HM	III	24	1 day 4 hours
31	INF	INF	II	35	1 day 17 hours
32	RDS BPD	HM BPD	III	648	3 months
33	IVH PH	IVH (PH)	I	64	2 days 17 hours
Moderate RD					
34	RDS	HM (PH)	—	1	16 days
35	IVH pneumoth	IVH (pneumothorax)	I	240	15 days
36	IVH	INF (IVH PH)	—	2	1 day 1 hour
37	INF RDS	INF (PH)	II	192	9 days
38	RDS	IVH	I	34	1 day 14 hours
39	RDS PH	HM	II	98	4 days
40	RDS IVH	HM (IVH)	I	23	5 days
Slight or no RD					
41	Apnea pneumoth	HM (pneumothorax)	II	236	18 days
42	IVH pneumoth	HM (IVH pneumothorax)	II	168	11 days
43	INF RDS pneumoth	INF (IVH PH)	I	31	1 day 13 hours
44	Apnea Inf sec	INF	I	609	26 days
45	INF IVH	INF (IVH)	I	72	7 days
46	INF	INF	I	130	8 days
47	Apnea Inf sec	INF	I	264	45 days
48	INF RDS	INF (IVH)	I	6	21 hours
49	IVH	IVH	I	1	1 day 5 hours

initial response to treatment was good, but was followed by gradual deterioration Despite survival of up to 45 days after prolonged IPPR, no evidence of BPD was found

Chest roentgenograms

The chest X rays in 9 cases of the *severe RD* group showed grade II or III diffuse reticulogranularity and air bronchograms (31) characteristic of severe RDS Two cases with grade I changes had brain haemorrhage, but no signs of pulmonary disease In the category of *moderate RD* two of the five chest X-rays obtained showed grade II changes, these 2 patients were diagnosed as clinically RDS Six of the eight X-rays in the category of *slight or no RD* did not show any reticulo granularity In the 4 cases with infection, infiltrations indicating pulmonary infection were seen only in the later phase

Autopsy findings

Pulmonary Table 2 shows the material divided into four groups on the basis of autopsy data

I Hyaline membrane group (HM) (Fig 1) Twelve cases were included in this group The extent thickness and degree of resolution of the membranes was variable, but otherwise lung morphology showed considerable similarities Some inflammatory cells both neutrophiles and

Table 2. Bacteriologic and microscopic findings in the 4 autopsy categories (see text)

Case no	Hyaline membranes: Distribution	Hyaline membranes: Thickness	Hyaline membranes: Resolution	Inflammatory cells: Intra alveolar	Inflammatory cells: Inter alveolar	Inflammatory cells: Intra bronchial	Inflammatory cells: Peri bronchial	Fibrinous material: Intra alveolar	Fibrinous material: Intra bronchial	Hemorrhages: Intra alveolar & bronchial	Hemorrhages: Inter alveolar peribronch	Focal necr	Bacterial findings
Hyaline membranes													
23										+ +	+	–	–
4										–	–	–	
25										–	–	–	
8										+	–	–	
29													
30			+							–		–	–
32											–	–	Klebsiella pn, E coli
34	+								–	+	+	–	–
39	+	+								+ + +	+ +	–	–
40									–	–	–	–	–
41	+									+	–	–	–
47		+								–	–	–	–
Infection													
26	–			+ + +	+	+	+	+	+	–	–	+ +	E Coli
31				+	+		+	+ + +	+	+	+	+ +	Klebsiella pn
36				+	+			+ +	+ + +	+ +	+ +	–	–
37				+ + +	+			+ +	+ +	+ +	+ +	+ + +	Klebsiella pn
43		–		+	+	+ +	+	+ + +	+ + +	+ +	+	–	–
44			–		–	+ +	–	+ +	+	–	–	–	Klebsiella pn
45				+ +		+	+	+ +	+ + +		+	+ +	E coli
46				+ +	+	+ +	+ +	+ +	+ + +	+	+	+	Pseudomonas
47				+		+		+	+			–	E coli
48		–	–	+ + +		+ + +	+	+ +	–		–	+ + +	Haemoph infl
PH													
33					–	–			–	+ +	+	–	–
HH													
27		–									–	–	
35											–	–	–
38											–	–	–
49		–							–			–	–

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3

4

Fig. 3. Case 45: the alveoli and bronchiolar lumina are filled with fibrinous material (grade + + +). Inflammatory cells are seen in the alveoli, the interalveolar septum and in the peribronchial tissue. HE × 170 magnification.

Fig. 4. *Case 4*: pneumonia with numerous focal necroses (+ + +). HE × 170 magnification.

1

2

Fig 1 Case 34 an alveolus lined with hyaline mem branes thickness + + and resolution + + Hematoxylin and eosin (HE) × 450 magnification

Fig 2 Case 42 an alveolus lined with hyaline mem branes thickness + + and resolution + + + In addition fibrinous material and inflammatory cells are in the alveolus HE × 450 magnification

lymphocytes were seen in 4 of the 12 cases The presence of intraalveolar inflammatory cells correlated with the extent of membrane resolution (Fig 2) Significant inter alveolar or peribronchial inflammation was not seen Case 32 showed pronounced intraalveolar inflammation and fibrosis as well as interalveolar septal fibrosis (Fig. 6) This case was clinically and radiologically diagnosed as suffering from bronchopulmonary dysplasia (18)

II Inflammation (INF) A histologic diagnosis of pneumonia was made in 10 cases (Fig 3) In 6 of these there were small focal necroses in the lung parenchyma (Fig 4) Neutrophilic and lymphocytic inflammatory cells were found in the interalveolar and peribronchial tissue as well as in the air spaces where a large amount of fibrinous material was also found Aspirated amniotic material was never found Bacteriologic investigation revealed gram negative bacilli *E coli Klebsiella* and *Pseudomonas* in 5 and *Haemophilus influenzae* in 1 of the cas s

In 6 of the 10 cases early rupture of the fetal membranes associated with foul smelling amniotic fluid at birth suggested intrauterine infection Death before the age of 48 hours in 3 of these (nos 26 31 48) was further evidence suggesting intrauterine infection In cases 36 and 43 copious amounts of fibrinous material were pres nt in the airspaces but no focal

Table 3 *Major pulmonary and associated post mortem findings in RDS (27 cases)*

Pulmonary finding	Associated finding	No of cases
Hyaline membranes (12 cases)	Massive IVH	3
	Massive PH	2
	None	7
Pneumonia (10 cases)	Massive IVH	3
	Massive IVH PH	2
	Massive PH	1
	None	4
Pulmonary hemorrhage	Massive IVH	1
No pulmonary abnormality	Massive IVH	4

Table 4 *Clinicopathological correlation in 27 cases of RDS*

Definitive diagnosis	Correct clinical diagnosis	Wrong clinical diagnosis	Missed clinical diagnosis	*Total definitive diagnosis*
RDS/HMD	10	4	2	12
Pneumonia	9	—	1	10
Massive IVH	9	—	4	13
Massive PH	2	2	4	6

necroses were seen and no organisms were found in bacteriologic investigation Clinically these cases had been diagnosed as having RDS but no hyaline membranes could be seen on histologic examination Thus despite the clinical history the histologic picture and the early deaths at 25 and 37 hours of age respectively suggested intrauterine or parenterally acquired infection

In the last 2 cases (nos 44 and 47) inflammatory cells were seen only within the air spaces and no focal necroses were observed Clinically these infants suffered severe apnoeic attacks when 15 and 55 hours old inflammatory symptoms only appearing after prolonged IPPR The age at death in these cases was 45 and 26 days which was considerably greater than in the other eight infectious cases

III Pulmonary hemorrhage (PH) Only 1 case (no 33) showed signs of a massive pulmonary hemorrhage as a single lesion of the lungs In this case the hemorrhagic area involved 20% of the total area of the sections In the HM cases pulmonary congestion was accompanied by microscopic hemorrhages of varying degree in 6 of the 12 cases (Fig 5)

In the infected lungs microscopic areas of hemorrhage anatomically contiguous were seen in 5 of the 10 cases 3 of whom also had focal necroses An additional case showed generalized intrapulmonary hemorrhage

IV No pulmonary lesions In 4 cases (nos 27 35 38 and 49) with massive intraventricular brain hemorrhage no pathologic features could be demonstrated in the lungs

Intraventricular brain hemorrhage A massive intraventricular brain hemorrhage (IVH)

Fig 5 Case 23 intra alveolar hemorrhage combined with hyaline membranes HE ×450 magnification

Fig 6 Case 32 bronchopulmonary dysplasia with fibrosis of the interalveolar septa HE ×450 magnification

were found in most cases of hyaline membranes or infection

Massive brain hemorrhage Of the 13 cases with this finding in only 4 were there no pulmonary abnormalities In these 4 cases the brain hemorrhage was considered the cause of respiratory failure In the remaining 9 cases the pulmonary abnormalities were considered to b the primary ones and accordingly they were classified on these findings

Pulmonary effect of IPPR and oxygen treatment There are reports that IPPR treatment may be harmful IPPR has been suggested to cause local lung damage (24 26 29) as well as circulatory changes which may predispose to IVH (1 7 25 28) High concentrations of oxygen may also produce acute pulmonary damage (14 18 22 23 24 25 29, 30) and in severe RDS Northway et al have sugg sted that it may result in later bronchopulmonary dysplasia" (18) Our results are compatible with Northway s view in that the sole severe case of RDS to survive 3 months showed these changes while l ss severe or infected cases did not On the other hand w did not s e any pulmonary lesions which have not been described in infants not treated with IPPR An unusual finding was the high incidence of IVH (5 of 8) in those infants with intra uterine infection however as this was not matched by a similarly high incidence in the RDS group IPPR treatment is unlikely to have b en an important cause of it.

SUMMARY

Tw nty seven deaths in premature infants weighing less than 1 250 g who were treated with intermittent positive pressure respiration are reported The major pulmonary abnormalities were hyaline membrane formation infection and hemorrhage occurring alone or in combination with intraventricular hemorrhage Hyaline membran s were associated with severe clinical features of respiratory distress whereas infection was not Prolonged treatment resulted in bronchopulmonary dysplasia in only 1 case where severe clinical respiratory distress was associated with the finding of hyaline mem branes at post mortem

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Correlation of clinical and autopsy findings

Table 4 shows the incorrect or missed clinical diagnoses There seems to be a tendency to overdiagnose hyaline membranes and pulmonary hemorrhage (4 and 2 cases respectively) whereas all cases diagnosed as having IVH and infection were confirmed at autopsy On the other hand, there were 4 cases of combined IVH and PH in which the diagnosis was not made before death

There was a good correlation between the severity of clinical RDS and the distribution and thickness of the pulmonary hyaline membranes found at autopsy Thus, cases allocated to the category of *severe RD* also had the most pronounced hyaline membranes (Table 1 and 2) The occurrence of massive IVH however was not correlated with the severity of the respiratory symptoms There were 2 cases of infection in the categories of *severe RD* and *moderate RD* and 4 cases in the category of slight or no RD

DISCUSSION

The present study reports the pathological findings in infants treated with IPPR The series could be divided into four categories on pathological criteria

Pulmonary hyaline membranes In this category hyaline membranes were practically the sole finding Other authors (11 13, 15 33) have stressed associated pulmonary vascular congestion but this, as well as gross hemorrhage, was less frequent in our cases Inflammatory cells were related to resolution of the membranes and could therefore represent a reaction to this process as has been suggested by others (13, 16 21, 32, 33, 24) The negative bacterial cultures in 11 of the 12 cases is evidence against an infective course for the lesion and furthermore, the cell distribution in this group and that with proven infection without membranes was different interstitial inflammation which Olding (10) considered essential to a diagnosis of bacterial infection, being rare in those with hyaline membranes alone

Chest radiography is one of the best diagnostic aids in neonatal respiratory disorders (3 10 12, 20) The degree of reticulo granularity on chest roentgenograms correlated well with the autopsy occurrence of pulmonary hyaline membranes (4, 9) However, minor degrees of hyaline membranes were found at autopsy in the less severe cases which had not shown these radiographic features

Pulmonary infection In 10 cases the pulmonary lesions were considered to be due to infection In 2 of these cases this may have resulted from prolonged IPPR Both showed limited fibrinous material and cell infiltration in the airspaces in both cases gram negative organisms were found In the remaining 8 cases 6 had a history of early rupture of the fetal membranes including 3 with evidence of criminal induction of labor In these cases, a characteristic finding seemed to be focal necroses in the lung parenchyma This specific abnormality has been attributed to gram negative bacteria (27) and in 5 of the 6 cases with this lesion such organisms were isolated All of them had much fibrinous material in the airspaces These findings are quite different to the diffuse inflammatory process with evidence of aspiration and little fibrinous material in the airspaces considered characteristic of neonatal pneumonia (6 17 27)

Pulmonary hemorrhage Extensive pulmonary hemorrhage unrelated to hyaline membranes or other pulmonary pathology is a common finding in newborn infants at autopsy (2) However as the sole lesion this was found in only 1 case in this series In the other cases (Table 3) it accompanied pulmonary hyaline membranes (2 cases) or infection (3 cases) In addition, restricted areas of microscopic hemorrhage

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RECONSTRUCTION OF OESOPHAGUS WITH COLONIC TRANSPOSITION

G GROTTE L. OLSEN and A REUTERSKIÖLD

From the Department of Paediatric Surgery University Hospital Uppsala Sweden

The replacement of defective parts of the oesophagus has always been a major surgical problem A large number of operative methods and modifications have been tried since Bircher (2) in 1894 performed the first oesophageal reconstruction using an antethoracic skin pedicle Some few methods have gradually crystallized and these are discussed below

Relatively few series of patients are reported in the Scandinavian literature where the whole or parts of the oesophagus have been replaced by colonic transplants We therefore present and discuss below a series of 6 children operated on during the last 4 years at the University Hospital Uppsala

MATERIAL AND METHODS

Of these 6 patients 5 had oesophageal atresia Three of these underwent acute operation with direct end to end anastomosis however sutural insufficiency necessitated ligation of the distal oesophagus and cervical oesophagostomy The other 2 with atresia had much too great a distance between the oesophageal ends to allow primary direct anastomosis The sixth child finally underwent operation for severe life threatening haemorrhages from oesophageal varices

CASE REPORTS

Patient 1 M G Oesophageal atresia with tracheal fistula Operation with end to-end direct anastomosis and closure of the fistula On the 4th day postoperatively there was leakage through the anastomosis and the distal oesophagus was therefore closed and cervical oesophagostomy performed Fed via gastrostomy and reoperated at the age of 14 months with retrosternal transposition of colon On the 10th day post operatively a cervical fistula developed but this healed spontaneously Subsequent course free of complications and the patient has now been followed up for 3 years and 8 months since the operation (see Table 1)

Patient 2 I B Oesophageal atresia with fistula to trachea Operation on the first day of life with end to-end anastomosis and division of the fistula After 3 days reoperation for leakage in the anastomosis with closure of the distal oesophagus and transposition of the proximal part to the neck Gastrostomy At the age of 13 months colonic transposition in the same way as in patient 1 Complication free course during the observation time of 3 years and 8 months (see Table 1)

Patient 3 J F Oesophageal atresia with tracheal fistula Acute operation with end to-end anastomosis and closure of the fistula On the 2nd day rupture of the thoracic wound necessitating resuture On the 5th day there was sutural insufficiency at the anastomosis and the oesophagus was therefore closed and cervical oesophagostomy performed on the proximal part At the age of 6 months colonic transposition was made as above Endotracheal intubation for bronchopneumonia with respiratory insufficiency 3 days postoperatively After a further 4 days a cervical fistula developed with considerable leakage Tracheostomy had to be performed After 2 1/2 months he was discharged from hospital with a fenestrated silver cannula After a further 3 months during which time he was looked after at home the cannula was removed with a good result Essentially free of complications during the subsequent observation period and it is now 3 years and 4 months since the operation (see Table 1)

Patient 4 T N Oesophageal atresia with tracheal fistula (from the proximal segment) The distal segment was very short and direct anastomosis was impossible The fistula was divided and the proximal part of the oesophagus was transposed to the neck Gastrostomy was performed Was then nursed at home

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esent symptoms
iffic swallowing solid food borborygmus n chest at times Loose stools sometimes nucous not ejectile
ccasional stomach pains Borborygmus (much ess than initially) Catches cold rel easily
ough Loose stools frequent 2/day and only slight subjective symptoms
iffic swallowing solid food if poorly chewed slight infections (upper respiratory) borborygmus
eartburn acid regurgitation retrosternal burning pain
el abundant muc secretion in throat Diff in learning to swallow

mal egment the transplant is sutured distally to the anterior side of the stomach on the fornix near to the lesser curvature Pyloroplasty according to the Finney technique is always performed as well as gastrostomy

RESULTS

Up to now the results in our series have been very encouraging No deaths have occurred and the first five patients are living an essentially normal life and have only minor discomforts The last patient has stricture tendency at the upper anastomosis but the observation time is to short to make a prognosis Two of the patients complain of difficulties in swallowing solid and inadequately chewed food Three of them are troubled by moderate borborygmus retrosternally while only one patient has had symptoms indicating reflux with heartburn etc (see Table 1) Finally 2 of the patients have loose stools but both have only relatively minor subjective symptoms

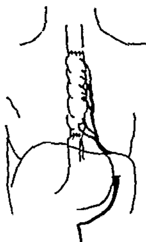


Fig 1 Technique according to Waterstone Colon segment here placed dorsally within the left pleural cavity Vascular pedical coming up dorsally through the diaphragm Lower colon anastomosis to oesophagus with preservation of cardia

DISCUSSION

Since Bircher (2) made the first reconstruction of the oesophagus with an antethoracic skin pedicle in 1894 (published 1907) a large number of modifications have been attempted with the aim of replacing anomalous or destroyed parts of the oesophagus

Kelling (5) for example performed the first colonic transposition in 1911

In Sweden Lundblad (8) performed in 1920 an antethoracic colonic transposition in a 3



Fig 2 Retrosternal extra pleural technique Lower colonic anastomosis to the ventricle

Table 1 *Summary of case reports*

Pat	Primary disease	Age at op	*Immediate postop complications*	Postop *obs time* years	The values are for all the patients within normal limits (height weight)	X ray
M G 640716	Oesoph atresia with fist to trachea	*14 mo*	*10th day cervical fistula (healed spontan)*	*3 8/12*	100 cm 16 kg	Good passag
I B 640820	Oesoph atresia with fist to trachea	13 mo	0	*3 8/12*	100 cm 15 5 kg	Good passag
J E 640729	Oesoph atresia with fist to trachea	18 mo	3rd day intub for resp *diff 7th day* cerv fistula Tracheostomy with fenest silver cannula 5½ mo	3 4/12	102 cm *15 kg*	(Not done)
T N 650923	Oesoph atresia with fist to trachea	13 mo	5th day cervical fistula Healed *spontaneously*	2 7/12	93 cm 12 5 kg	Good passag
S W 570226	Banti s disease with oesoph varices	10 4/12 years	0	1 11/12	135 cm 25 kg	Moderate constriction but good passa
A K 680508	Oesophageal atresia No fistula to trachea	8 mo	Bilateral pneumothorax immediately postop 7th day cerv fistula healed spontaneously	4/12	70 cm 8 1 kg	Moderate co good passage striction at upper anastomosis Pass satisfactory

and had serval attacks of upper respiratory infection and gradually also *left sided* exudative pleuritis which healed without complications Operation with colonic transposition at the age of 13 months the *same technique being used as in the above patients* On the 5th day a cervical fistula developed from the proximal anastomosis 3 1/2 months postoperatively there was a sudden onset of difficulty in swallowing with vomiting attacks but no pathological changes were observed in the transplant Relatively rapid and spontaneous improvement and now 2 years and 7 months after the operation the patient is in a good general state of health (see Table 1)

Patient 5 S W At the age of just under 2 years there was an onset of *melaena After* extensive investigations liver cirrhosis with portal hypertension was diagnosed Partial gastrectomy was performed *1 1/2* years later local ligation of varices in the cardial region was performed After one further year of severe haemorrhages a spleno renal shunt was carried out Also after this operation there were repeated haemorrhages from large oesophageal varices At the age of 9 1/2 years an anastomosis was made between the vena cava and the superior mesenteric vein Despite this the haemorrhages recurred and the transfusions became increasingly difficult because of the child having pronounced hypersensitivity reactions At 10 years and *4 months* the lower part of the oesophagus and half of the stomach were resected and about 1 dm of transverse colon was transposed to the ga thus produced No further bleeding One year and 1 months has now elapsed since the operation and th patient is in a good general condition (see Table 1

Patient 6 A K Born with oesophageal atresi with no fistula to the trachea and with a large distanc between the oesophageal segments Direct anasto*mosis technically impossible* Fed by gastrostomy Attempts for several months at dilating the proxima pocket with a rubber bougie gave no results an finally colonic transposition was performed at th age of 8 months Rather complicated postoperative course and artificial ventilation was necessary fo 1 week A cervical fistula occurred on the 7th day but healed spontaneously The observation time is now only 8 months and it is therefore too early to make a prognosis

Operative technique

We have used the retrosternal technique in a one stage procedure as follows

The ascending and transverse colon are mobilized and a pedicle with the middle colic artery is utilized The caecal pole which is usually too thick to allow *a good cervical anastomosis is resected The trans*plant which in 5 of the cases mainly comprised the ascending colon and in 1 case the transverse colon is then placed isoperistaltically through a retrosternal tunnel After end to end anastomosis with the proxi

Louhimo et al (7) have experienced diffi culties for their children in gaining weight They did not use pyloroplasty in any of six cases which may th n be of importance in this con nection

Age As regards a suitable age for the ration Gross & Firestone (3) consider that patient should be at least one year old Both terston (15) and Rehbein et al (11) consider optimal age to be about 6 months for the son that the thorax can then be easily reached ough an incision at the level of the sev h thoracic vertebra and that the vascular tem at that age is sufficiently well developed a good pedicle to be obtained

Othersen & Clatworthy (9) on the other nd prefer to wait until the age of about 18– months since the child can then be expected sit relatively steadily and reflux after feeding thereby prevented. Linder & Hecker (6) also ns der the best age to be during the second ar of life

Three of our patients were about 1 year of e at the time of operation One patient was nly 6 months old another was 8 months and ne just over 10 years (see Table 1) The most roublesome symptoms postoperatively occurred n the 6 month-old child We consider that the most suitable age for operation is about 1 year Theoretically it should be po sible however to perform the op ration earlier since at least metabolically an earlier operation should be well tolerated Immunologically there is probably no difference of importance from about 6 months of age und upwards neither as re gards humoral nor cellular immunity especially concerning coli organisms

For patients with oesophageal atresia we consider that the operation should not be de ferred much later than the age of 1 year be cause of the great inconveniences of feeding through a gastrostomy for a long period

Iso- or antiperistaltically? Linder & Hecker (6) place their transplants both iso- and anti peristaltically and Gross & Firestone (3) do the same Waterston (15) uses mainly isoperistaltic colon however Othersen & Clatworthy (9) consider that it is of no significance how the intestine is placed in this respect Of consider ably greater importance is the blood supply to the intestine the length of the transplant and its diameter etc

We have placed the colon isoperistaltically in all cases and consider that this question most probably is of no importance for the results

The few experimental investigations that have been made indicate that the isoperistaltic in testine functions somewhat more efficiently than the antiperistaltic The peristalsis is very slow, however Sherman & Waterston (14) have observed deep peristaltic waves of the colonic type in contraststudies Other investigators e g Schafer (13) have al o demonstrated oscilla tory concertina like movements roentgenologi cally Rehbein et al (11) have observed both these types of peristalsis

In our opinion however peristalsis plays a very minor part in this connection and the most important factor is the force of gravity— which has also been pointed out by Othersen & Clatworthy (9)

Gastrostomy We have performed gastros tomy in all cases and there seem to be no divided opinions on this question among other authors

Stricture—bouginage? Rehbein et al (11) discuss the tendency of the transplant to be come dilated postoperatively They attribute this partly to the fact that the transplant was too long from the start partly to a functional stenosis in the distal anastomosis due to the difference in calibre and finally to the effect to reflux (in op rations by the retrosternal tech nique) He therefore dilates the distal anasto mosis postoperatively by means of a contin uous silk thread which is placed in position during the operation

This stricture can often however be only a relative constriction as an expression of the intrathoracic dilation of the colon (due to the negative intrathoracic pressure) and the intra abdominal constriction (due to positive intra abdominal pressure) If roentgenography shows an apparent passage of food and the patient

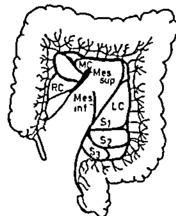


Fig 3 Showing right (*RC*) left (*LC*) and middle (*MC*) colic arteries and sigmoid arcades S1–3 (according to *Griffiths 1956*)

year-old child with a good result, Sandblom (12) made an attempt at intrathoracic colonic transposition in a 2 day old child in 1947 and Pettersson (10) performed the first successful intrathoracic colonic transposition in a newborn (1-day old) child in 1959

Most authors seem to agree that the colon has greater advantages as a replacement for the oesophagus than, for example the small intestine or mobilization of the stomach At present in principle two methods are used, in which a part of the colon is interposed between the proximal oesophagus and distal oesophagus or stomach (Figs 1 and 2) The colon can either be placed retrosternally (as recommended by Gross & Firestone (3) among others) or it can be placed in the left pleural cavity behind the root of the lung (as performed by Waterston (15)) In this clinic the former method has been used

Waterston (15) and Rehbein et al (11) use transverse colon isoperistaltically on a pedicle with the left colic artery They make the distal anastomosis with the distal oesophagus and thus preserve the cardia

Sherman Jr (14) on the other hand uses ascending colon isoperistaltically by the retro sternal technique

Gross & Firestone (3) also favour the retrosternal technique, which is technically simpler, gives better lung function postoperatively and, most important of all, it keeps the pleural cavity clean in the event of leakage At present they prefer the descending colon, they consider that this has the advantage that a longer segment can be mobilized We have had no technical problems, however, in getting the ascending colon, lying isoperistaltically on a pedicle with the middle colic artery to be of sufficient length

Linder & Hecker (6) describe 5 cases where they used either ascending transverse or descending colon with the retrosternal technique (Fig 3)

In the first 5 cases we resected the caecal pole In the last patient however, we retained the caecum and divided the colon just above Bauhin s valve The reason for this was that two of the patients had been found on follow up to be having loose stools One possible explanation for this may be that Bauhin s valve has an inhibitory effect on the propulsion through the intestines and that caecal resection would thus result in too rapid propulsion with consequent diarrhoea However according to recent investigations by Johansson & Nylander (4) Bauhin s valve has no such action its function being to prevent the contents of the colon from passing in a retrograde direction The diarrhoea might instead be explained by an infection ascending to the small intestine We therefore intend to retain the caecal pole in future cases

We consider that another detail of great importance is that the transplant should be made as straight as possible i e without kinks, especially in the distal part so as to prevent obstruction This has also been pointed out by Rehbein et al (11) who have tried to make the transplant so short that it lies like a rubber band under tension' Gross & Firestone (3) are also of a similar opinion

In agreement with most authors we also perform pyloroplasty in order to facilitate evacuation of the stomach postoperatively This may be of great importance in preventing reflux of acid gastric juice up into the transposed colon which may result in ulceration bleeding and even perforation

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has no discomfort, it is therefore our firm opinion that bouginage should be avoided Gross & Firestone (3) also advise against bouginage and instead suggest reoperation with revision of the anastomosis in the event of postoperative obstruction

In none of our 6 patients was bouginage of the distal anastomosis necessary Patient 6, however, has a tendency to stenosis in the proximal anastomosis, which requires bouginage postoperatively

Reflux One of the main differences between the retrosternal and the intrathoracic technique is that in the latter case the distal anastomosis is made between the colon and oesophagus immediately above the cardia, the function of which is then retained Waterston (15) aimed at preserving the function of the cardia in his series which, however, meant a higher operative risk However, in a large number of his patients he does not seem to have obtained satisfactory cardial function (personal communication)

One difficulty with Waterston's (15) technique is to produce a hole in the diaphragm large enough so as not to have any pressure effect on the pedicle, with a risk of necrosis of the transplant, but not so large that herniation can occur

In spite of the relatively large number of operations that have now been performed by the retrosternal technique strikingly few cases have been reported with peptic changes in the transplant due to reflux (Battersby 1)

Further, with the retrosternal technique we have had no major problems of the reflux oesophagitis type Only one of the patients (S W) has had symptoms of this, and they have been very mild We have observed no ulceration in the colonic transplant on postoperative roentgenography

Cervical fistulae Cervical fistulae are a common complication according to most authors Gross & Firestone (3) reported 6 fistulae all of which healed, in their series of 47 patients and Othersen & Clatworthy (9) reported 4 fistulae in 11 patients

Of our 6 patients, 4 had cervical fistulae on the 5th to 10th day, all of which closed spontaneously, however, and have since caused no trouble to the patients

Operative risk The average mortality in some of the larger series with colonic transposition, performed for benign diseases in the oesophagus is around 10%

The series reported here is small and there is no mortality

SUMMARY

With the increased experience which has been gained during recent years, transposition of the colon can be regarded as a valuable method when for various reasons it is desirable to replace the whole or parts of the oesophagus

A series of 6 patients operated on by the retrosternal technique with good results is reported

On the basis of this series the advantages and disadvantages of this method are discussed and compared with other types of replacement of the oesophagus by colonic transposition

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Table 1 *Clinical data of our patients suffering from ataxia telangiectasia*

Pat no	Age in years	Age at the onset of cerebellar ataxia (years)	Age at the onset of ocular tel angiectasia	Skin sings	Infections	Other members of family with ataxia telangiec tasia
1 Sz Zs	14	4	Newborn period	Cutaneous telangiectasia vitiligo lentigo	Repeated pneu monia bronciectasia chronic otitis vulvitis	—
2 L D	10	Infancy	Newborn period	Cutaneous telan giectasia café au lait spots follicu lar hyperkeratose lentigo	0	—
3 J P	10	1½	5 years	Follicular hyperkeratose	0	Sister
4 L H	11	6	5 years	Cutaneous telangiec tasia cafe au lait spots follicular hyper keratose lentigo	Repeated pneu monias chronic otitis pleuri tis	—
5 S Sz	9	3	1½ years	Cutaneous telangiectasia	Frequent common cold pneumonia two times otitis	—
6 (Sz	13	9	1 y ar	Café au lait spots follicular hyperkeratose lentigo	Repeated pneumo-nias chronic otitis bronchiectasia urogenital infection	Sister
7 F T	10	3	4 years	Cutaneous telan giectasia lentigo	Chronic otitis chron ic mastoiditis sinu sitis maxillaris	Sister

for provocation (1 100 in negative cases) Aisen bergs (1) or Young s (45) technique was employed for the measurement of DNCB skin reactions Intra dermal tuberculin tests were performed with an 1 10 dilution of tuberculin PPD and *in* cases of positive reaction another test with an 1 1 000 dilution fol lowed The reactions were read after 72 hours

The clinical data of our patients are shown in Table 1.

RESULTS

The rates of blastic transformation and the mi totic indices (number of mitoses per one thou sand cells) are to be found in Table 2 the val ues listed represent the results obtained from cultures containing AB Rh positive serum In cubation with the patient s own serum gave values for both blastic transformation and mi totic index slightly lower but not significantly different from the results with AB Rh positive serum Generally blastic transformation and mitotic index were found to be depressed in ataxic patients but reached statistical signifi cance only in respect to the number of mitoses However in ca e 3 neither the blastic trans formation rate nor the mitotic index was dif ferent from that of the controls This can be seen in the partial overlapping of the values too In one of our cases (Zs Sz) four examina tions were performed in 2 years where the de gree of the depression of blastic transforma tion proved to vary in the course of the disease (Table 3) No correlation was found between the plasma IgA levels or the results of the de layed type skin reactions and the decreases in blastic transformation No correlation ap-peared between these values and the occur rence of chronic recurrent infections either (Table 2)

The incorporation of ^{3}H thymidine also

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LYMPHOBLASTIC TRANSFORMATION, CHROMOSOME PATTERN AND DELAYED TYPE SKIN REACTION IN ATAXIA TELANGIECTASIA

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The syndrome of conjunctival vasodilatation and cerebellar ataxia was first described in 1941 by Louis Bar (25) and was called ataxia telangiectasia The frequent occurrence of sino-pulmonary infections in this condition was pointed out by Boder & Sedgwick (3), and Centerwall & Miller (4) independently in 1958 The diminution or lack of serum IgA level was first demonstrated on a large material by Thieffry et al in 1961 (42) In 1963 Peterson et al (29) suggested that the immunological deficiencies in patients with ataxia telangiectasia may be related to abnormal thymic function This suggestion has been subsequently confirmed

A decreased blastic transformation of lymphoid cells was observed by Leikin et al (23) and has been confirmed by numerous authors (17, 20, 21, 24, 27, 28) In one of our patients, however, we found lymphocytic blastic transformation to be almost normal (37), we therefore repeated this examination on six further cases

MATERIAL AND METHODS

Blastic transformation was studied in peripheral leucocyte cultures by the slightly modified method of Moorhead (26 36) The total volume of each culture amounted to 10 ml/7 42 ml TC 199 (Difco) culture medium+2 0 ml human plasma+0 5 ml cell suspension+0 08 ml phytohaemagglutinin+1 0 mg streptomycin+10 000 E penicillin Incubation wa[s] carried out both with AB Rh positive plasma an[d] with the patient s own plasma After 72 hours 0 0[5] ml of a 0 04 % colchicin solution was added to eac[h] culture after 2 hours harvesting hypotonisation i[n] 0 5 ml TC 199+2 0 ml distilled water fixation (wit[h] acetic acid+methanol) and flaming and drying wer[e] then performed The morphological feature distin[-]guishing lymphoblasts was a large loosely structure[d] nucleus with occasional nucleoli nontransforme[d] cells were characterized by intensely stained nearl[y] black amorphous bodies (15) Transitional forms wer[e] classified as blasts In every culture 1 000 cells wer[e] counted by each of three examiners

Chromosomal studies were carried out only i[n] well defined metaphases Since in ataxic individual[s] the number of mitoses is reduced chromosomal eval[-]uation could be performed in only 4 cases in 11[?] cells together

The incorporation of ^{3}H thymidine into nuclea[r] DNA was studied in 3 cases To the nutrient mediu[m] (2 5 ml) consisting of TC 199 (Difco) and 20 A[B] Rh positive human plasma 0 02 ml of phytohaemag[-]glutinin P was added Twenty four hours before har[-]vesting 1 μC of ^{3}H thymidine (specific activity 1[?] C/mM) in a volume of 0 1 ml was added to th[e] cultures After 24 hours the cultures were centrifuged the sediment was washed sequentially with TC 19[9] concentrated acetic acid and methanol and the pre[-]cipitate was dried at 37 C Radioactivity was meas[-]ured by liquid scintillation in a Packard apparatus Incorporation of H thymidine was examined 24 48 96 and 120 hours after cultivation was started

To examine the delayed type skin reaction Can[-]dida skin test was performed with an intracutaneou[s] administration of 0 05 ml of 1 1 000 dilution o[f] stock solution (Institute Pasteur Paris) and checke[d] after 48 hours In skin tests with 2–4 dinitrochloro[-]benzole a 10 solution was used for sensitizatio[n] and a 1 1 000 dilution of the 10 stock solutio[n]

Table 5 *Chromosomal studies in ataxia telangiectasia*

Patient no	Total no of counted and analysed cells	No of cells with chromosome count of <45	45	46	47	No of breaks	Breaks percent	Other structural aberrations
1 Zs Sz	38	1	3	32	2	4	10	—
2 D L	30		1	29		1	3	—
3 P J	27	2	1	24		—	—	—
4 H L	15	1	2	12		1	6	—

35) As can be seen from Table 6 even one and the ame individual may show different reactions if examined with different antigens It is generally agreed that lymphoblastic transformation is the best method at present to study the cellular immunity the procedure requires however a reliable technique Although a decrease in the blastic transformation was observed in all ataxic patients it was variable Data in this respect in the literature is shown in Table 7 Similarly to our findings it can be seen that values found in ataxic cases by the authors listed under 2 3 and 4 reached and even exceeded the lower limit of the controls

Differences are presumably due to errors of the morphological method (34) In ataxia telangiectasia there are numerous transitional cell forms which some authors classify as non transformed lymphoid cells (18) some others however describe them as transformed cells

Registration of the mitotic index seems to be much more reliable and it yielded greater difference in our cases than the morphological evaluation of blastic transformation did even though it has also the disadvantage of being based on sampling i e it is never possible to core all the cells in the cultures We agree with Naspitz et al (27) that the best method is the evaluation of ^3H *thymidine uptake* In our cases the latter method revealed a considerable decrease in DNA synthesis

There was no decrease of blastic transformation in our first case (Zs Sz Table 2) in 1966 (37) The same patient died 2 years later in con equence of a pyloric tumor (colloid carcinoma) In the course of these 2 years the lymphocytes of the patient were cultured three more times and each examination revealed a decreased blastic transformation of varying degree The different results may be due to a change in the degree of inhibition of blastic transformation but it may as easily be attributed to the inexactness of morphological evaluation

IgA deficiency is sometimes associated with chromosomal aberrations The chromosome 18 seems to be affected in most cases (either deletion or partial trisomy) but D/D translocation

Table 6 *Delayed type skin reaction in ataxia telangiectasia*

Patient no	Tuberculin reaction	PPD	Candidin (1 1 000)	2-4 dinitro-chlor benzol (1 1 00)
1 Zs Sz	1 10 negativ	—	Negativ	Negativ
2 D L	1 100 negativ	1 1 000 negativ	Negativ	±
3 P J	1 100 10 10 mm	1 1 000 negativ	Negativ	±
4 H L	1 100 negativ	1 1 000 negativ	Negativ	—
5 Sz S	1 10 negativ	—	Negativ	±
6 Sz G	1 1 000 5 8 mm	1 1 000 negativ	Negativ	+

Table 2 *Lymphoblastic transformation and mitotic index in six patients with ataxia telangiectasia*[a]

Patient no	Percentage of blastic transformation		Mitotic index		IgA (serum)	Delayed type skin reactions[b]	Infections
	Patient	Control	Patient	Control			
1 Zs Sz	60 7	70 9	0 4	1 1	No	Decreased	+
2 D L	79 6	87 6	0 3	1 1	No	Decreased	0
3 P J	75 9	79 1	0 7	0 5	No	Slightly decreased	0
4 H L	75 9	87 5	0 1	0 9	Decreased	Decreased	+
5 Sz S	74 1	80 4	0 1	2 8	Normal	Decreased	±
6 Sz G	67 9	79 3	0 3	1 8	Normal	Slightly decreased	+
Mean and SD	$X = 72\ 3 \pm 6\ 87$	$X = 80\ 8 \pm 6\ 23$	$X = 0\ 3 \pm 0\ 22$	$X = 1\ 7 \pm 1\ 01$			
	60 7–79 6	70 9–87 6	0 1–0 7	0 5–2 9			
Ranges	$p = 0\ 5$		$p < 0\ 01$				

[a] The morphological examination of blastic transformation was not made in our patient 7 because repeated culturing was not possible owing to the lack of parents approval
[b] See Table 6

showed a decrease in DNA synthesis in ataxic patients In the cultures of controls ^{3}H thymidine incorporation reached the peak after 96 hours and in ataxic subjects after 120 hours (Table 4)

Results of chromosome studies are listed in Table 5 In 1 case (Zs Sz) the number of breakages slightly exceeded the upper limit of values registered in the controls while in the other cases numerical or structural aberration breakage or signs of translocation could not be found Findings of delayed type skin reactions are shown in Table 6

DISCUSSION

Ataxia telangiectasia is a clinical syndrome characterized by cerebellar ataxia and oculocutaneous telangiectasia These symptoms are associated with immunologic abnormalities which vary from patient to patient IgA for instance is usually absent from plasma, although in certain cases (including some of ours) the plasma IgA concentration may be perfectly normal (2, 8, 16 19, 24, 31, 40) The common bacterial antibody level was likewise variable (9 13, 29, 30) As regards their delayed type skin reactions, patients with ataxia telangiectasia behave again variously, while the reactions is usually weak, normal reactions have also been registered (3, 16 24, 30 31

Table 3 *Results of studies of lymphoblastic transformation during 2 years in patient 1*

Zs Sz	Blastic transformation	Mitotic index
1 (1966 V)	68 3	1 1
2 (1966 XI)	54 2	0 1
3 (1966 XII)	60 3	0 3
4 (1968 III)	60 2	0 1

Table 4 *Lymphoblastic transformation estimated by ^{3}H thymidine incorporation Counts per minute incorporated*

	Ataxia teleangiectasia				Normal
	P J	T F	Zs Sz	Mean	
24 h	1 375	1 134	1 616	1 375	1 679
48 h	1 834	1 200	3 764	2 333	11 901
96 h	21 756	23 137	36 052	26 982	62 523
120 h	24 027	31 465	30 681	28 724	33 966

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Registration of the mitotic index seems to be much more reliable and it yielded greater dif ference in our cases than the morphological evaluation of blastic transformation did even though it has also the disadvantage of being based on sampling i e it is never possible to core all the cells in the cultures We agree with Naspitz et al (27) that the best method is the evaluation of ^{3}H thymidine uptake In our cases the latter method revealed a consid erable decrease in DNA synthesis

There was no decrease of blastic transforma tion in our first case (Zs Sz Table 2) in 1966 (37) The same patient died 2 years later in consequence of a pyloric tumor (colloid car cinoma) In the course of the e 2 years the lymphocytes of the patient were cultured three more times and each examination revealed a decrea ed blastic transformation of varying de gree The different results may be due to a change in the degree of inhibition of blastic transformation but it may as easily be attri buted to the inexactness of morphological eval uation

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5 Sz S	1 10 negativ	—	Negativ	±
6 Sz G	1 1 000 5 8 mm	1 1 000 negativ	Negativ	+

Table 7 *Literary data concerning the morphological examination of lymphoblastic transformation in ataxia telangiectasia*

Patient no	Authors	No of cases	Per cent of blast cells taken the controls values to 100 Patients	Per cent of blasts Controls	Ranges of blasts Patients	Ranges of blasts Controls
1	Leikin S L et al 1966 (23)	5	37 8	74 0	20 4–46 6	63 6–84 5
2	Lévêque B et al 1966 (24)	4	39 2	75 0	0–75	75–
3	Oppenheim J J et al 1966 (28)	5	34 0	88 0	0–91	23–93
4	Hayakawa A & Kobayashi N 1967 (20)	2	55 5	80 3	34 6–76 8	74 1–91 6
5	Gotoff S P et al 1967 (17)	1	23 2	75 0	17 5–29 0	60–90
6	Gropp A & Flatz G 1967 (18)	2	22 2	89 0	20 0–23 5	88–90
7	*Our patients*	*6*	*72 3*	*80 8*	*60 7–79 6*	*70 9–87 6*

has been found too However, numerous authors (5, 10 12 32, 33, 41 43) have not found any chromosomal abnormality Increased spontaneous chromosome breakage was observed by Jacobs et al (22) in Swiss type agammaglobulinaemia by Hecht et al (21) and by Gropp & Fratz (18) in ataxia telangiectasia The latter finding can have a connection with the well known increased frequency of leukaemia and lymphoreticular tumours in ataxia telangiectasia (3, 6 7, 14 17 21 31) In contrast to our patients suffering from Fanconi s anaemia (38) it was only in one of the cytogenetically analysable cases of ataxia that the number of breakages slightly exceeded the control values this was the very patient who developed malignancy (Table 5) No breakage or other chromosomal aberration was observed in any of the other subjects Normal chromosome patterns were found also by Utian (44), Zellweger (46) Schuster et al (39) and Young et al (45) in ataxia telangiectasia It seems that contrary to the decreased blastic transformation, increased breakage of the chromosomes is not a constant feature of this syndrome

SUMMARY

In 6 patients suffering from ataxia telangiectasia morphological evaluation of lymphoblastic transformation was performed, in one of these cases it was repeated four times In 3 cases the incorporation of ^3H thymidine was measured to examine the degree of the lymphoblastic transformation Both methods revealed decreased lymphoblastic transformation though with the last method the decrease was much greater No correlation was found between the degree of decreased blastic transformation the infections and the plasma IgA level Delayed type skin reactions were subnormal in varying degrees Increased frequency of chromosome breakage was observed in 1 case—who developed malignancy and died—but no chromosomal aberrations were found in the three other cytogenetically examined patients

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Table 7 *Literary data concerning the morphological examination of lymphoblastic transformation in ataxia telangiectasia*

Patient no	Authors	No of cases	Per cent of blast cells taken the controls values to 100	Per cent of blasts	Ranges of blasts	
			Patients	Controls	Patients	Controls
1	Leikin S L et al 1966 (23)	5	37 8	74 0	20 4–46 6	63 6–84 5
2	Léveque B et al 1966 (24)	4	39 2	75 0	0–75	75–
3	Oppenheim J J et al 1966 (28)	5	34 0	88 0	0–91	23–93
4	Hayakawa A & Kobayashi N 1967 (20)	2	55 5	80 3	34 6–76 8	74 1–91 6
5	Gotoff S P et al 1967 (17)	1	23 2	75 0	17 5–29 0	60–90
6	Gropp A & Flatz G 1967 (18)	2	22 2	89 0	20 0–23 5	88–90
7	Our patients	6	72 3	80 8	60 7–79 6	70 9–87 6

has been found too However, numerous authors (5, 10 12 32 33 41, 43) have not found any chromosomal abnormality Increased spontaneous chromosome breakage was observed by Jacobs et al (22) in Swiss type agammaglobulinaemia by Hecht et al (21), and by Gropp & Fratz (18) in ataxia telangiectasia The latter finding can have a connection with the well known increased frequency of leukaemia and lymphoreticular tumours in ataxia telangiectasia (3 6 7 14, 17 21, 31) In contrast to our patients suffering from Fanconi s anaemia (38) it was only in one of the cytogenetically analysable cases of ataxia that the number of breakages slightly exceeded the control values this was the very patient who developed malignancy (Table 5) No breakage or other chromosomal aberration was observed in any of the other subjects Normal chromosome patterns were found also by Utian (44) Zellweger (46) Schuster et al (39) and Young et al (45) in ataxia telangiectasia It seems that contrary to the decreased blastic transformation increased breakage of the chromosomes is not a constant feature of this syndrome

SUMMARY

In 6 patients suffering from ataxia telangiectasia morphological evaluation of lymphoblastic transformation was performed in one of these cases it was repeated four times In 3 cases the incorporation of ^{3}H thymidine was measured to examine the degree of the lymphoblastic transformation Both methods revealed decreased lymphoblastic transformation though with the last method the decrease was much greater No correlation was found between the degree of decreased blastic transformation the infections and the plasma IgA level Delayed type skin reactions were subnormal in varying degrees Increased frequency of chromosome breakage was observed in 1 case—who developed malignancy and died—but no chromosomal aberrations were found in the three other cytogenetically examined patients

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THE BEHAVIOUR OF SOME INDICES OF CALCIUM PHOSPHATE METABOLISM IN TURNER S SYNDROME

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In many individuals with Turner s syndrome osteoporotic changes may be seen in the skeleton Theoretically these changes may be caused by a disturbance in calcium phosphate metabolism Little data is available in the literature on this problem It is not possible to decide on the basis of the available data to what exten these disturbances are due to the basic disease and to what degree a superimposed disorder is responsible for them Some interesting observations made on patients with Turner s syndrome who had short metacarpal and metatarsal bones suggest that the calcium phosphate disturbances found are similar to those of pseudohypoparathyroidism (3 15)

The aim of this study is to evaluate some indices of calcium phosphate metabolism and renal regulation of acid base balance in individuals with Turner s syndrome

MATERIAL AND METHODS

Clinical radiological hormonal and cytogenic studies were performed on a group of 6 patients with Turner s syndrome aged 10 to 18 years In 1 patient (case 6) there were symptoms typical of rickets (hypophosphatemia radiological signs of rickets in bones) (5)

After establishing the diagnosis calcium and phosphorus balance studies were carried out The excretion of calcium and phosphate was determined in faeces collected for 3 consecutive days For 3 days preceding and during the faeces collection the patients were kept on a constant diet containing 650 mg of calcium and 950 mg of phosphate per day The amounts of calcium and phosphate administered were calculated from the tables of their contents in foodstuffs after comparing them with the data obtained from direct determinations in Department of Human Nutrition (Poznan) Following the balance studies in intravenous calcium loading test (15 mg/kg b w over 4h ours) was performed (4 13) An intravenous parathormone loading was performed modified as follows on the first day urine was collected from 8 a m to 2 p m blood was taken at 11 a m On the second day 200 I U parathormone (Lilly) was administered at 8 a m as a 3 hour intravenous infusion urine was collected and blood was taken as on the first day This modification was introduced in order to avoid difficulties connected with diurnal changes in calcium and phosphorus (1)

Calcium phosphate and creatinine concentrations in blood and urine were determined during each of the loading tests Calcium was determined with a flame photometer phosphate by Fiske Subbarow method and creatinine by Folin Wu method The clearance of phosphate (Cp) was determined in ml/min/1 73 m and reabsorption of phosphate in renal tubules (TRP) was calculated as a percentage of the amount filtered

The ability of the kidney to maintain acid base balance was evaluated by ammonium chloride test as described by Greder & Guttman (2) giving orally 0 1 g NH Cl per kg of body weight The excretion of titrable acidity the ammonium ion excretion and the total excretion of hydrogen ion before and after loading were determined

RESULTS

Clinical data and the results of radiological cytogenetic and renal function studies are presented in Table 1 Table 2 shows indices of calcium phosphate metabolism and parameters of renal acid base balance The results ob

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Dilution and concentration of urine	Sex chromatine	Karyotype
001 1 078	Neg	45/X0
003- 1 030	Neg	45/X0
003- 1 077	Neg	45/X0
007- 1 077	Neg	45/X0
007 1 030	Neg	—
007 10 5	Neg	45/X0

3 cases in 3 cases slightly raised (cases 3 5 and 6) Tubular reabsorption of phosphate (TRP) in 3 patients approached the lowest normal values in 2 patients slightly (cases 4 and 5) and in 1 patient distinctly lowered (case 6)

After calcium loading (Fig 1) the serum calcium and phosphate levels behaved normally (13) Cp was decreased considerably in 4 cases (cases 1 3 4 and 5) TRP increased distinctly only in 2 cases (cases 3 and 6) After Parat hormone loading (Fig 2) no rise in serum calcium level was observed except in 1 patient (case 6) in 1 patient no decrease in serum phosphate occurred (case 1) and in 1 (case 6) a rise was observed Cp increased distinctly in 4 cases (cases 1 3 4 and 5) TRP decreased only insignificantly in 5 cases in 1 case even slightly increased (case 6)

Hydrogen ion excretion expressed either as titrable acidity or total excretion of hydrogen ion (Fig 3) was decreased both before and after NH_4Cl loading Similarly the ammonium ion excretion was decreased in all the patients except in case 5

No exact correlation was found between the disturbances in the calcium phosphate metabolism indices studied and the magnitude of

...cretion of H before and after H Cl in / Eq min 1 73 m²

...itrable ...cidity	Total excretion
3 7 7 3	36 8 58 [illegible]
4 7 7 [illegible]	39 3 57 3
2.1 1 1	30 5 69 8
4 6 6 1	37 4 81 8
[illegible]00 3 1	[illegible] 08 1
0 8 2.3	30 4 71 5
5 5 8 7	103 5 177 3

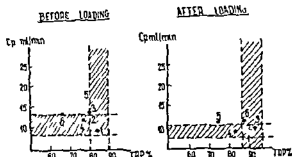


Fig 1 Influence of calcium loading on phosphate excretion in Turner's syndrome ▨ normal values

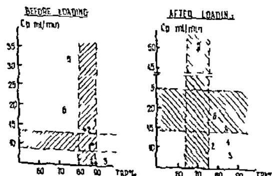


Fig 2 Influence of parathormone loading on phosphate excretion in Turner's syndrome ▨ normal values

Table 1 *Clinical and laborator) data in indu iduals with Turner's syndrome*

Case	Age in y	Height cm (deficit)	X ray of bones	Alkaline phosphatase u Bod	Ccr (ml/min/ 1 73 m²)	Electrolytes in urine Na (mEq/kg/24 h)	K	Ca	P (mg/kg/24 h)
1	18	146 (11)	Osteoporosis Brachydactyly IV V metatars	5 4	95	2 8	0 9	0 08	18
2	16	137 (14)	Osteoporosis Brachydactyly IV metacarp	4 7	75	3 1	0 9	0 11	15
3	15	128 (19)	Osteoporosis	5 7	55	3 3	0 7	0 07	14
4	14	135 (15 °)	Osteoporosis Brachydactyly IV metatar	4 4	85	3 8	1 1	0 06	19
5	14	125 (20)	Osteoporosis	5 5	105	3 1	0 7	0 09	17
6	10	110 (25)	Osteomalacia Signs of rickets in the epiphyses	6 7	80	2 8	0 6	0 09	13
					Normal values	3 5 ±1 3	0 9 ±0 4	0 09 ±0 04	16 ±4

tained were compared (Figs 1–3) with the values found in 10 healthy children aged 8–14 years

In all subjects studied the calcium level in blood was normal, the phosphate level was normal in 4 patients in 1 slightly lowered (case 5) and in 1 distinctly lowered (case 6) Calcium *excretion in faeces exceeded the amounts in*gested in 4 cases (cases 1, 2, 3 and 6) and in 2 cases was normal Phosphate excretion in faeces was normal

Clearance of phosphate (Cp) was normal in

Table 2 *Indices of calcium phosphate metabolism in Turner s syndrome*

	Balance			Calcium loading In serum		In urine		Parathormone loading In serum		In urine	
Case	Ca ( )	P ( )		Ca (mEq/l)	P (mg)	Cp (ml/min)	TRP ( )	Ca (mEq/l)	P (mg)	Cp (ml/min)	TRP ( )
1	−23	+62	Before	4 6	4 3	9 7	86 6	4 69	4 4	10 3	89 9
			After	6 3	4 8	7 2	90 1	4 79	4 4	14 4	86 3
2	−40	+48	Before	4 7	5 1	10 5	85 7	4 84	4 8	14 8	84 6
			After	5 4	5 8	8 2	88 1	4 84	4 3	13 8	79 9
3	−14	+52	Before	4 7	4 3	13 8	80 0	4 73	5 8	5 8	92 8
			After	6 7	5 1	10 0	92 4	4 92	4 9	8 7	85 4
4	+30	+44	Before	4 6	5 2	12 1	77 4	4 63	5 5	4 4	91 8
			After	5 6	5 9	9 1	83 6	4 80	4 4	15 8	81 0
5	+30	+66	Before	4 7	3 3	16 2	79 9	4 70	3 4	31 9	74 3
			After	4 9	4 3	11 2	74 8	4 78	2 3	52 5	71 0
6	−26	+78	Before	4 9	2 8	13 2	67 0	4 90	3 1	20 2	72 0
			After	5 9	3 8	11 4	88 5	5 50	4 4	20 5	78 8
Mean Normal values			Before	4 7	4 2	12 7	78 0	4 80	4 6	10 5	87 0
			After	5 3	4 8	6 1	91 0	5 00	3 9	24 2	73 0

activity of peripheral organs to parathyroid hormone reminiscent of the findings in pseudo-hypoparathyroidism may play a certain role in the pathogenesis of observed changes in the o seous system and of the deficiency of growth The diminished ability of the kidney to excrete the excess of acid may play also a significant role in a growth failure A po itive correlation was ob erved between the deficit of height and the diminution of hydrogen ion excretion

SUMMARY

The results of calcium and parathormone load ing tests in 6 individuals with Turner s syn drome indicate a certain degree of insensitive ness of the tissues especially of the kidney when compared with healthy individuals A similar insensitiveness expressing it elf by a decrease in hydrogen ion excretion was found after ammonium chloride loading An analysis of the results suggest the existence in this syn drome of insensitiveness of renal tubules to regulatory factors Osteoporosis and stunting of growth may be connected partly with in testinal calcium loss occurring at puberty and partly with this insensitiveness to regulatory factors

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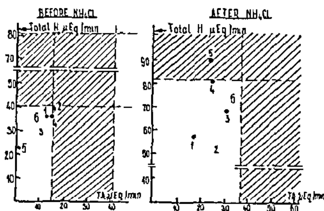


Fig 3 Hydrogen ion excretion in Turner's syndrome
▨ normal values

growth deficiency Neither was it possible to show any precise correlation between the degree of osteoporosis and the presence of other bony changes such as shortening of metacarpal and metatarsal bones in the cases 1 2, 4, and the variations of the indices of calcium-phosphate metabolism Similarly no correlation was observed between the increased calcium excretion in faeces and the abnormal results of load tests The result obtained suggest only a certain correlation between the age and the increased calcium excretion in faeces in the oldest girls (cases 1, 2 and 3) An increased calcium excretion in faeces in the youngest girl (case 6) may be connected, at least in part to the disturbances associated with rickets (14)

There was however, sufficiently marked positive correlation between the rate of reduction of hydrogen ion excretion expressed both as titrable acidity and total excretion and the degree of growth deficiency

DISCUSSION

The nature of the disturbances in calcium phosphate metabolism in Turner's syndrome has not as yet been elucidated

In the present investigations, the calcium and phosphate levels in blood were normal The pronounced hypophosphatemia in case 6 is probably due to coexisting rickets (5) Virtually no changes were observed in Cp, TRP, or phosphate excretion in urine, the only exception being a very low degree of TRP in case 6 mentioned above

The calcium loading tests were in part in conformity with the results obtained in normal subjects (4 13) In all the cases there was a rise in calcium and phosphate levels in blood in 4 cases there was adistinct decrease in Cp But in only 2 cases was a more pronounced increase in TRP found (Fig 3) The parathormone loading revealed a diminished end organ sensitivity to this hormone, especially as expressed in terms of alterations of TRP

The reason for the above mentioned disturbances in calciumphosphate metabolism indices is still obscure There may be a diminished reactivity of peripheral tissues to regulatory factors especially to parathyroid hormone Changes of this kind may affect growth and skeletal formation process as, on one hand they diminish the transport of phosphate to cell (9, 11 12), and, on the other hand, they reduce the exchange of calcium and phosphate within the skeleton (8 10)

The investigation on calcium balance revealed *an increased calcium excretion in faeces* in the oldest subjects studied The attempts to find the cause of this phenomenon were unsuccessful This negative calcium balance in Turner's *syndrome however, may* be one of the causes making pubertal growth acceleration impossible

Abnormal kidney function was observed while investigating its role in regulation of the acid base balance The decreased hydrogen ion excretion expressed as titrable acidity found before and after ammonium chloride loading proves some impairment of efficient work of nephron *in this respect Ammonium* ion excretion although not shown in Table 2 was also low except for case 5 (hence a higher total hydrogen ion excretion in that case) Other disturbances in renal function have previously been observed in the cases of Turner's syndrome (6 7)

On the basis of our investigations the function of parathyroid glands *in the Turner's syn*drome appears to be normal The changed re-

Table 1 *Patient data for three children on regular haemodialysis treatment*

Patient	Age at the start (years)	Body weight at the start (kg)	Duration of treatment (months)	No of treatments	Diagnosis
1	14	25 5	25	221	Chronic pyelonephritis vesico-ureteral reflux
2	13	32.5	15	131	Chronic pyelonephritis vesico-ureteral reflux
3	7	18 0	2	16	Hereditary renal disease congenital nephrosis

sexual development with normal menstruations has occurred

Prior to dialysis only patient 2 was hyper tensive during treatment all are normotensive without drugs Patients 1 and 2 were nephrectomized for control of urinary tract in fection

None of the patients had clinical signs of neuropathy Signs of hyperparathyroidism or other abnormality in calcium metabolism have not been noted

An arteriovenous fistula may cause over growth of the affected limb if the metaphyses are *not* closed This phenomenon is a classical part of the syndromes of Klippel Trenauney and Parkes Weber The present patients dis played *no signs of hypertrophy* or increased skel tal growth on physical or roentgenological examination possibly due to the fact that the fistula is made from a very distal artery to a superficial vein *There* were no clinical signs of insufficient nutritive blood flow to the fi stula arm

In patient 1 cardiac outpu*t cen* a*l* venous pressure and arterial pressure with open and closed fis ula were measured as well as the total blood volume (Table 2) The difference in cardiac output with open and closed fistula was less than 10% We could find no signs of adverse effect upon the heart and the blood volume was within normal limits ECG and chest X ray examinations have shown no in dication of cardiac decompensation

All the patients are emotionally well ad justed to dialysis treatment Their general con dition has much improved and the repeated percutaneous cannulations have given them no emotional problems Patient 1 has now fin ished her schooling and is working full time the days she is not on dialysis Patient 2 has returned to school between the dialyses They have been able to use their arms without re-strictions and they do not care about the pres ence of a fistula

We have found the Cimino-Brescia sub-cutaneous direct arteriovenous fistula well suited for haemodialysis *in children* of this age We have found no signs of circulatory over load during the time of observation and the risk of overgrowth of the arm carrying the fi stula is probably overrated

Table 2 *Haemodynamic data for patient 1*

Length 144 cm weight 8 kg blood volume 2,5 l hae matocrit 26

	Cardiac output (l min)	Arterial pressure (mmHg)	Central venous pressure (mmHg)
Fistula open	6 7	130/70	–1
Fistula closed	6 3	133/75	–2

SUMMARY

The subcutaneous fistula according to Cimino-Brescia was used in three children 14 13 and 7 years of age for regular haemodialysis up to 25 months No adverse effects on the central or local circulation were recorded and no sig-nificant difference appeared in the growth of the fistula limb compared with that of the con tralateral The repeated percutaneous punc

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ARTERIOVENOUS FISTULA FOR HAEMODIALYSIS IN CHILDREN

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Regular haemodialysis is getting more and more common even in children, most of whom are prepared for kidney transplantation In most cases reported, access to the patient s vascular system has been given by an external arteriovenous shunt according to Quinton et al (11) using a prosthesis of teflon and silastic (3, 5, 6 7, 9 12 13) Distal arteries, e g the radial artery or posterior tibial artery, have been cannulated in older children (>30 kg) the brachial, axillary or femoral arteries in smaller children and infants This external type of shunt has some disadvantages risk of infections, coagulations, skin necrosis, bleedings etc , and the patients are incapacitated by its presence

Arteriovenous fistula as described by Brescia et al (4) is often used in the adult and has eliminated many of the disadvantages of the Quinton-Scribner technique In children the subcutaneous fistula has been avoided due to the supposed risk of complications with cardiac overload and hypertrophy of the limb carrying the fistula (5, 12)

We have tried the subcutaneous fistula in three children on regular haemodialysis

METHODS

Surgical technique We have used the technique described by Brescia et al (4) with some modifications (10) The anastomosis was made side to side between the radial artery and the cephalic vein on the non dominant left arm and all four limbs of the vessels were left open

Dialysis technique Metal cannulas gauge 14-16 were used for puncture of the veins and were connected to the Alwall Gambro disposable dialyzer (1 2) using a blood pump In the two older children the normal 11 layered model for adults was used but the smallest child was treated with a 6-layered model (8) Treatments were given 4-8 hours twice weekly

Roentgen examinations were performed on the chest (heart size overhydration) and both forearms (length of radius and ulna bone thickening or sclerosis)

CASE MATERIAL

Three children 14 13 and 7 years of age had chronic renal failure with endogenous creatinine clearance values less than 5 ml per minute per 1 73 m² and were selected for regular haemodialysis in preparation for renal homotransplantation (see Table 1)

RESULTS AND DISCUSSION

The fistula veins were used for dialysis 2 days after operation in patient 1 In the other two patients we made the fistulae weeks before dialysis treatment was necessary Cannulations of the veins were made by dialysis nurses and have given very slight technical problems No major haematoma or local infection occurred at the site of the cannulations

All the patients were underweight and smaller than normal children when dialysis treatment was started The two elder patients in whom treatment has continued long enough for evaluation have both grown, and in patient 2

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REVIEW ARTICLE

ARTERIAL ANOMALIES CAUSING COMPRESSION OF THE TRACHEA AND/OR THE OESOPHAGUS

A Report of 30 Symptomatic Cases

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Anomalies of the aortic arch and its branches are fairly common (6 12) Most of these anomalies are asymptomatic They are diagnosed at autopsy by mere chance with radiological examination of the trachea and oesophagus or at angiocardiography for other congenital cardiovascular malformations Symptomatic cases are rare the symptoms being caused by compression of the trachea and/or the oesophagus Symptoms may arise from anomalies not only of the aorta and its branches but also from an aberrant left pulmonary artery or from an aneurysm of the pulmonary artery (5) In our experience the prevalence of symptomatic cases among other congenital cardiovascular malformations is less than 0 5% The symptoms caused by arterial derangements within the chest may be life threatening and therefore early diagnosis and surgical treatment are important in these symptomatic cases

The literature on these anomalies is considerable including aspects on embryology anatomy symptoms diagnosis and surgery (2 4–14) The aspects on the diagnostic aids and surgery have however been somewhat contradictory and it is the aim of this cooperative study to evaluate the diagnosis and treatment of arterial anomalies with such severe symptoms from compression of trachea and/or oesophagus that surgery has been indicated and to report the long term postoperative results

There are many different types of vascular rings and other arterial anomalies causing similar symptoms and there is a great variability within the special group The following classification is confined only to those anomalies found in our material They do however constitute the most common types (see the schematic figure Fig 1)

1 *Double aortic arch* The two arches encircle the trachea and the oesophagus

2 *Right aortic arch with left ligamentum arteriosum or patent ductus arteriosus* The ligamentum or the ductus completes the ring by passing from the left side behind the oesophagus to the aorta

3 *Aberrant right subclavian artery* arteria lusoria The vessel arises as the last branch from the aortic arch and passes to the right side of the chest usually behind the oesophagus but may also pass between the oesophagus and the trachea or in front of the trachea

tures have given no significant technical or psychological problems to the patients nor to the nurses

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Table I Vascular Ring cases double aortic arch

Case no	First symptom age	Indication for surgery	Operation Age	Operation Year	Surgical procedure division of	Postoperative course	Additional anomalies
1	1 mo	Constant stridor + recurrent resp infect	5 y	1950	Anterior arch	Uneventful	
2	1 wk	Increasing stridor + recurrent resp infect	18 mo	1951	Posterior arch + PDA	Stridor first months	
3	1 wk	Constant stridor	2 mo	1950	Ant arch + left subclav artery	Tracheostomy Decannulation after 18 mo	
4	1 wk	Increasing stridor	2 mo	1954	Post arch + lig art	Tracheostomy died 1 wk after operation	
5	1 wk	Increasing stridor + recurrent resp infect	6 mo	1955	Post arch + lig art	Cyanotic attacks for 1 mo after operation	
6	1 wk	Increasing stridor	3 mo	1958	Ant arch + PDA	Tracheostomy + respirator Died 4 wk after operation	Atrial septal defect primum type
7	3 mo	Increasing stridor	3 mo	1961	Post arch + lig art	Uneventful	
8	1 wk	—	10 days	1958	Ant arch	Tracheostomy + respirator Died 2 days after operation	Tracheo oesophageal fistula
9	1 wk	Stridor + cyanotic attack with unconsiousness	6 mo	1959	Post arch	Tracheostomy + respirator	
10	1 wk	Increasing stridor	6 mo	1959	Left common carot art	Tracheostomy + respirator Died 24 days after operation	
11	1 mo	Dysphagia + stridor	4 mo	1965	Ant arch	Stridor for 1 year after op	
12	1 wk	Reflex apnea	13 mo	1967	Ant arch + lig art	Tracheostomy + respirator 1 week	
13	1 mo	Increasing stridor	18 mo	1968	Post arch + lig art	Uneventful	
14	1 wk	Increasing stridor	6 mo	1967	Post arch + PDA	Tracheostomy + respirator Decannulation 18 mo after operation Slight stridor 3 y after operation	

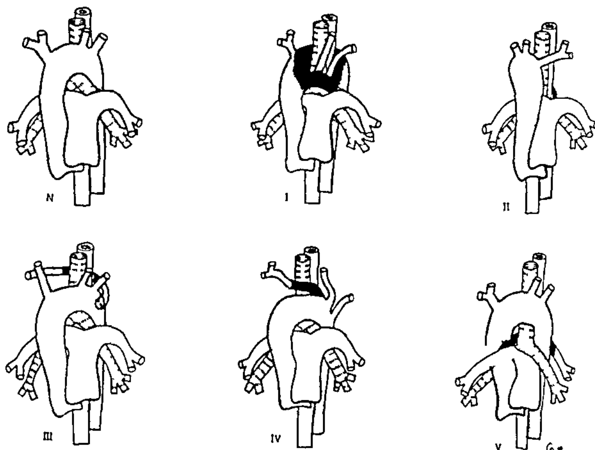


Fig 1 Schematic drawings showing the various vascular anomalies Black areas show the compressing part of the vessels N normal anatomy I double aortic arch II right aortic arch with left ligamentum arteriosum III aberrant right subclaviar artery IV anomalous innominate artery V anomalous left pulmonary artery

4 *Anomalous innominate artery* The artery originates from the aortic arch distally and passes in front of the trachea to the right side of the neck

5 *Anomalous left pulmonary artery* The bifurcation of the pulmonary artery is displaced to the right and the left pulmonary artery passes posteriorly turning around the right superior tracheobronchial angle to the left lung between the oesophagus and the trachea

CLINICAL MATERIAL

The material consists of 30 cases 16 boys and 14 girls which were seen during a period of 19 years from 1950 to 1968 Five cases have previously been reported (2 3) The individual data are given in Tables 1-5 Double aortic arch was present in 14 cases right aortic arch with left ligamentum arteriosum in 5 aberrant right subclavian artery in 4 anomalous innominate artery in 4 and anomalous left pulmonary artery in 3 cases respectively Associated cardiovascular malformations were diagnosed in 7 patients and other significant malformations were diagnosed in another 5 cases Two infants had oesophageal atresia and another case had tracheo oesophageal fistula Anal atresia and recto urethral fistula was observed in one child with anomalous pulmonary artery Three children with arteria lusoria were mentally retarded one of them had Downs syndrome

SIGNS AND SYMPTOMS

In all children the symptoms which could be referred to the vascular anomaly started before the age of 5 months In 18 cases the onset of symptoms occurred already during the first week of life Ten out of these 18 cases had double aortic arch (Table 1) *Stridor* and other

Table 1 *Vascular Ring cases double aortic arch*

Case no	First symptom age	Indication for surgery	Operation Age	Operation Year	Surgical procedure division of	Postoperative course	Additional anomalies
1	1 mo	Constant stridor + recurrent resp infect	5 y	1950	Anterior arch	Uneventful	
2	1 wk	Increasing stridor + recurrent resp infect	18 mo	1951	Posterior arch + PDA	Stridor first months	
3	1 wk	Constant stridor	2 mo	1950	Ant arch + left sub lav artery	Tracheostomy Decannulation after 18 mo	
4	1 wk	Increasing stridor	2 mo	1954	Post arch + lig art	Tracheostomy died 1 wk after operation	
5	1 wk	Increasing stridor + recurrent resp infect	6 mo	1955	Post arch + lig art	Cyanotic attacks for 1 mo after operation	
6	1 wk	Increasing stridor	3 mo	1958	Ant arch + PDA	Tracheostomy + respirator Died 4 wk after operation	Atrial septal defect primum type
7	3 mo	Increasing stridor	3 mo	1961	Post arch + lig art	Uneventful	
8	1 wk	—	10 days	1958	Ant arch	Tracheostomy + respirator Died 2 days after operation	Tracheo-oesophageal fistula
9	1 wk	Stridor + cyanotic attack with unconsiousness	6 mo	1959	Post arch	Tracheostomy + respirator	
10	1 wk	Increasing stridor	6 mo	1959	Left common carot art	Tracheostomy + respirator Died 24 days after operation	
11	1 mo	Dysphagia + stridor	4 mo	1965	Ant arch	Stridor for 1 year after op	
12	1 wk	Reflex apnea	13 mo	1967	Ant arch + lig art	Tracheostomy + respirator 1 week	
13	1 mo	Increasing stridor	18 mo	1968	Post arch + lig art	Uneventful	
14	1 wk	Increasing stridor	6 mo	1967	Post arch + PDA	Tracheostomy + respirator Decannulation 18 mo after operation Slight stridor 3 y after operation	

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Table 2 *Vascular Ring cases Right aortic arch with left ligamentum arteriosum or persistent ductus arteriosus*

Case no	First symptom age	Indication for surgery	Operation Age	Operation Year	Surgical procedure division of	Postoperative course	Additional anomalies
15	1 wk	Recurrent respir infection	16 mo	1954	Lig art	Uneventful	
16	1 mo	Attacks of cyanosis	7 mo	1955	PDA + left subclavian	Attacks of cyanosis Died 1 wk after op	Ventricular septal defect Malformed tricuspid valves
17	3 mo	Dysphagia + stridor	4 mo	1959	PDA	Uneventful	
18	4 mo	Increasing dysphagia + attack of cyanosis	18 mo	1962	Lig art	Uneventful	Atrial septal defect primum type
19	1 wk	Recurrent respir infection	18 mo	1964	Lig art	Uneventful	

Table 3 *Vascular Ring cases anomalous right subclavian artery (arteria lusoria)*

Case no	First symptom age	Indication for surgery	Operation Age	Operation Year	Surgical procedure division of	Postoperative course	Additional anomalies
20	1 mo	Dysphagia + convulsions of unknown etiology	3 mo	1955	Art lusoria + PDA	Uneventful	Mental retardation + PDA
21	1 wk	Dysphagia	4 y	1967	Art lusoria	Uneventful	Mental retardation
22	1 wk	Respir infections + dysphagia	6 mo	1966	Art lusoria	Uneventful	Down s syndrome
23	1 mo	Dysphagia + recurrent respir infections + stridor	8 mo	1968	Art lusoria + PDA	Uneventful	PDA + slight valvular pulmonary stenosis

Table 4 *Vascular Ring cases anomalous innominate artery*

Case no	*First* symptom age	Indication for surgery	Operation Age	Year	Surgical procedure division of	Postoperative course	Additional anomalies
24	5 mo	Stridor	8 mo	1955	Innominate *artery*	Uneventful	Oesophageal atresia earlier operated
25	1 wk	Recurrent *broncho pneumonias*	7 mo	1964	Fixation *of the* innominate artery to sternum	Uneventful	
6	1 wk	Stridor recurrent resp infection	5 mo	*1965*	Fixation of the innominate artery to sternum	Stridor first weeks	Oesophageal atresia earlier operated
27	1 wk	Recurrent broncho pneumonias	6 mo	1968	Fixation of the innominate artery to sternum	Stridor a *few* weeks	

Table 5 *Vascular Ring cases aberrant left pulmonary artery*

Case no	First symptom age	*Indication* for surgery	Operation Age	Year	*Surgical procedure* division of	Postoperative course	Additional anomalies
28	3 wk	*Increasing stridor +* attacks of cyanosis	5 mo	1966	Transposition *of left pulmonary* art	Died 1 day after operation	Anal atresia + recto urethral fistula earlier operated
29	*1 wk*	—	—	*1967*	—	—	Aplasia *of left* pulm *inf* lobe Respirator 1 week before death
30	2 mo	Attacks of cyanosis + respir insufficiency Emphysema	5 mo	*1968*	Transposition of left pulmonary art	Tracheostomy + respirator Decannulation after *8 mo* Reoperation *after 3 mo* (thrombosis left pulm art)	

respiratory difficulties were observed in all but 2 cases The stridor was usually of inspiratory type accompanied by expiratory wheezing Belly cough and hoarse voice were common findings Tachypnea and other signs of respiratory distress usually occurred The respiratory difficulties were accentuated during increased activity such as feeding and crying *Recurrent respiratory infections* were prominent symptoms in 20 cases of all types During these infections the respiratory difficulties were aggravated to such an extent that in several cases an emergency operation was performed Eleven patients *had dysphagia* This symptom was not present in any of the cases with anomalous innominate or anomalous left pulmonary artery *Attacks of cyanosis* occurred in all the different types and was followed by episodes of unconscious ness in 2 cases with double aortic arch

In most cases the diagnosis was made probable or confirmed by means of *roentgenological examination* of the chest including contrast fill ing of the oesophagus In 8 cases the diagnosis was not disclosed at the first roentgenological examination of the chest In 5 cases this could be explained by the fact that contrast filling of the oesophagus had not been performed and in 3 cases, an inadequate examination could explain the lack of precision in the diagnosis Angiography was performed in 17 cases to con firm the diagnosis and to clarify the anatomy

The roentgenological findings in double aortic arch and right aortic arch with left liga mentum arteriosum or patent ductus arteriosus were an indentation and dislocation of the oesophagus and the trachea usually at the level of the third or fourth thoracic vertebra

The cases with aberrant right subclavian artery showed a posterior defect of the oesophagus and no tracheal compression

The roentgenological findings in anomalous innominate artery were visible in the lateral projection with an indentation in the anterior wall of the trachea just below the suprasternal notch The appearance of the oesophagus was normal

The findings in anomalous left pulmonary artery were a displacement of the lower pa of the trachea to the left and forward, and a indentation on the right main bronchus ne its origin There was also an indentation in th anterior wall of the oesophagus at the lev of the carina and an increased distance betwee the oesophagus and the trachea

OPERATIVE TREATMENT AND RESULT

Twenty-nine cases were operated In doubl aortic arch the smaller arch was divided In cases the ligamentum arteriosum was cut a well and in 1 case, the left subclavian arter was divided In right aortic arch a patent duc tus arteriosus was divided in 3 cases and liga mentum arteriosum in 2 In all cases of arteria lusoria the artery was divided and, in 2 cases a patent ductus arteriosus as well The anom alous innominate artery was divided in 1 case and sutured to the inside of the sternum in the other 3 cases Finally, in 2 cases, the anom alous pulmonary artery was divided and anas tomosed to the main pulmonary artery or to the left branch The third case of anomalous pulmonary artery (case 29) was not operated This girl had respiratory difficulties from birth As the symptoms were moderate and the right lung roentgenologically normal, surgical inter vention was not considered necessary for the moment At the age of 4 months she caught a respiratory tract infection with severe symp toms She was admitted for operation but was in such a bad condition that only tracheostomy could be performed During this procedure an extensive tracheal stenosis was found She did not improve and died a week later in a respirator

Postoperative complications were common in patients with double aortic arch and occurred in both patients with anomalous left pulmonary artery Persisting and even increased stridor and excessive tracheo-bronchial secretions made tracheostomy necessary in 10 cases and 9 out of these were treated with respirator Four of these 10 cases survived after respirator treat ment and decannulation could be performed in

1 case after a few days and in the three other not earlier than between 9 and 18 months after the operation

There were 8 deaths including the one not operated upon In the two children with anomalous left pulmonary artery autopsy showed a generalized tracheal stenosis Five children with double aortic arch died postoperatively All of them were tracheostomized and treated in respirator The left common carotid artery instead of the anterior arch was accidentally divided in case 10 One boy no 4 who had tracheal infection died after 1 week Malformed tricuspid valves with signs of severe incompetence were found in case 16

It should be noted that all postoperative deaths except one occurred during the first period of time 1950–1959 In the series of 15 cases operated between 1960 and 1968 only one child died after operation

Information of the postoperative course has been collected in all cases Most of the patients have been investigated with roentgenological examination of the chest including a contrast filling of the oesophagus The postoperative follow up time varies between 9 months and 19 years

In 7 patients the symptoms disappeared immediately or during the first month following surgery Mild stridor or dysphagia was present in 6 patients during a period up to 5 years after operation Two children had repeated episodes of bronchitis 1–2 years after surgery A mentally retarded girl no 20 died 1 year after a successful operation of arteria lusoria The cause of death was bronchopneumonia

A mild compression and dislocation of the trachea was present in 4 patients years after operation and slight dislocation of the oesophagus was seen as long as 8 years postoperatively

DISCUSSION

Many conditions may cause stridor in early infancy and have to be considered in the differential diagnosis of vascular anomalies The stridor in arterial anomalies may not be apparent until the age of several months and therefore not only the congenital conditions but also the acquired have to be included in the diagnostic considerations The most common congenital stridor is probably caused by soft tracheal wall or a flaccid epiglottis This condition as well as other structural anomalies of the larynx and the trachea may be verified by means of laryngoscopy Macroglossia micrognathia thyroid cysts or ectopic struma laryngitis bronchitis foreign bodies tumors and metabolic disorders associated with hypocalcemia are other disorders which could give stridor like symptoms In all these conditions the oesophagogram is normal Contrast filling of the oesophagus is the main diagnostic aid to the diagnosis of double aortic arch right aortic arch with left ligamentum aberrant right subclavian artery and anomalous left pulmonary artery Roentgenographic examination of the trachea gives an important contribution to the diagnosis of the last mentioned condition and of anomalous innominate artery The roentgenological appearance could however be less apparent in early infancy and therefore repeated examination could be necessary before the diagnosis is settled From surgical point of view angiography may be indicated giving more anatomical details especially when associated congenital heart lesions are suspected Lobar emphysema may also be suspected as in one of our cases of anomalous left pulmonary artery The emphysema comprised the whole right lung, however which is not seen in lobar emphysema Bronchoscopy and tracheography seems to be of comparatively little diagnostic value These investigations are dangerous in infants with respiratory difficulties and should in our opinion be avoided

The respiratory symptoms in the material published here have been referred to the vascular anomaly In a few cases with associated lesions however it may seem to be difficult to say definitely that the vascular anomaly was the main cause of the symptoms Mental retardation associated cardiac malformations and conditions after operation for oesophageal

atresia could give symptoms similar to those of vascular anomalies After studying the hospital records, we still believe that the vascular malformation has been the main cause of the respiratory difficulties except in one or two questionable cases

The operative risk should be weighed against the natural history of the disease and the post operative results The follow-up study of this material shows that the long term results are excellent even if the improvement may take years in a few cases Mustard et al (10) reported a material of 23 children with symptomatic vascular compression who were followed for 6 months to 7 years During that time 2 children died because of the vascular anomaly Twelve children however, were asymptomatic at the time of follow up A high mortality was reported in cases with aberrant left pulmonary artery (9) Out of 44 cases, 16 unoperated symptomatic children died and only 6 were asymptomatic Double aortic arch and anomalous left pulmonary artery are most apt to give symptoms demanding surgery Right aortic arch with left ligamentum arteriosum often needs operation while surgery is very seldom needed in anomalous innominate artery and in arteria lusoria Thus, even if the symptoms in some cases may subside children with symptomatic vascular compression run a great risk and if surgery is postponed they must be kept under close observation and surgery should be serious ly and repeatedly considered

The aim of the operation is to relieve the compression The operative technique varies from case to case as the details of the mal formation are never identical

An interesting and controversial detail in the surgical procedure is how far the dissection should be carried out around the trachea and the oesophagus

According to Mustard et al (10) the dissec tion should be minimal because the soft trachea needs all supporting tissue in order not to col lapse Gross (4) on the other hand speaks in favour of an extensive dissection to remove all sheaths and fibrous band that accompany the vessel Lincoln et al (7) who recently pub lished a series of 29 cases describe that 'Dissec tion around the trachea and oesophagus was made in all cases to divide all constricting fascial bands No untoward effects of such a procedure are mentioned Our series does not give support to one or the other opinion We think that fibrous rings and sheaths should be divided with minimal dissection

In children with double aortic arch and with anomalous left pulmonary artery the postopera tive course is rarely uneventful Difficulties are encountered especially in children with early symptoms Out of 10 children with double aortic arch and with symptoms during the first week of life, 8 needed tracheostomy and res pirator treatment postoperatively and 5 in the early series died The complications in these children were not correlated to the age at opera tion

From the material presented here, it is evi dent that the operative risk has been greatly reduced during the most recent years This is explained by increased experience in the sur gical technique and by improved postoperative care The relatively low operative mortality of today is a strong argument in favour of surgical treatment of arterial anomalies causing compression of the trachea and the oesophagus These anomalies may be life threatening and therefore early diagnosis is imperative The presence of persistent congenital stridor should warrant radiological investigation of the trachea and the oesophagus

SUMMARY

The experiences of diagnosis and treatment of arterial anomalies causing compression of the trachea and/or the oesophagus in 30 symp tomatic cases are reported The main diagnostic aid is the radiological examination of the chest in the anterior posterior and lateral projection including adequate contrast filling of the oesoph agus This investigation, using water-soluble contrast media, should be performed in infants with stridor and other respiratory difficulties

and in dysphagia With improved surgical technique and postoperative care the operative risk of today is low and surgery should be seriously considered in symptomatic cases Operation may be necessary as an emergency treatment

Addendum Another nine infants have been operated with no mortality since this material was collected Three cases had double aortic arch five right aortic arch with left ligamentum arteriosum and one infant had anomalous innominate artery

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and in dysphagia. With improved surgical technique and postoperative care the operative risk of today is low and surgery should be seriously considered in symptomatic cases. Operation may be necessary as an emergency treatment.

Addendum. Another nine infants have been operated with no mortality since this material was collected. Three cases had double aortic arch, five right aortic arch with left ligamentum arteriosum and one infant had anomalous innominate artery.

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SHORT COMMUNICATION

PERITONEAL DIALYSIS IN THE TREATMENT OF HYALINE MEMBRANE DISEASE OF NEWBORN PREMATURE INFANTS

Results of a Controlled Trial
Preliminary Report

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In our previous animal experiments (3) we *found a markedly increased tolerance against* otherwise lethal hypoxia in newborn rabbits and adult mice treated by peritoneal dialysis (PD) These results can be considered as supporting data on the pathologic effect of metabolites accumulated in the body fluids as additional humoral factors inhibiting the life of the cells On the other hand on the basis of these experiments we regarded as permissible to carry out therapeutic experiments *in the cases* of hyaline membrane disease (HMD) of newborn premature infants which failed to show improvement with the alkali glucose treatment

In such cases the PD has therefore been employed in an attempt to achieve the following therapeutic effects 1) correction of meta*bolic disturbances, including metabolic acidosis*, without the administration of excessive quantities of fluids and electrolytes and 2) elimination of possible toxic metabolic products that may accumulate during hypoxia

MATERIAL AND METHODS

A total of 92 premature infants with HMD have been studied 50 received PD (*dialysis group*) and 42 were *treated* by conventional methods (alkali glucose infusion according to Usher (5) the dose of bicarbonate adjusted to the results of pH and B E values of arterialised capillary blood determined by Astrup s method (1) (*control group*)

An infant was included in the series only when it failed to improve during treatment with alkali and glucose 1) for 4 or more hours when its clinical condition was classified according to Weisser s criteria (6) as grade III or 2) for 8 or more hours when its condition was classified as grade II

Inclusion in either the *dialysis* or control groups was then decided by drawing lots Conventional therapy was continued in the *control* groups and PD was instituted in the *dialysis group* by simultaneous discontinuation of any other treatment

Intermittent PD was performed in our first 38 cases The dialysis fluid contained 95 mEq Na^+ 4 0 mEq K^+ 4 0 mEq Ca^{++} 2 0 mEq Mg^{++} 105 mEq Cl^- and 15 glucose per litre Depending on the severity of the acidosis 40 to 50 mEq/l of sodium bicarbonate was added to the solution immediately before its use The solution contained penicillin (100 000 I U /l) but no heparin

Continuous PD was introduced beginning with the case *39* when a *suitable method had been devised* The apparatus for continuous PD is shown in Fig 1 Since the introduction of the continuous PD 100 I U *heparin per litre has also been added to the solution* From this time on the exchange of dialysis fluid with a rate of 120–150 ml/hour has usually been undisturbed and the outflow only rarely impeded The dialysis was maintained for 36 to 48 hours depending on the pattern of clinical symptoms In a single case it lasted for 72 hours

For better care of PD treated newborn before and during the dialysis the following parameters were controlled bodyweight serum Na and K concentrations total serum proteins chest roentgenogram

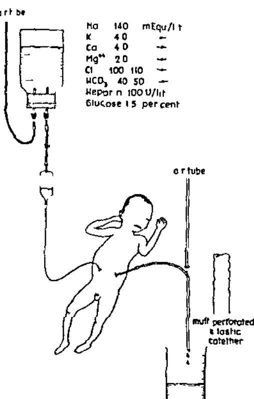


Fig 1 Apparatus for the continuous method of PD

Standard bicarbonate pH Pco of the arterialized capillary blood was monitored according to Astrup (1) twice daily Measurements directly concerning PD (abdomen circumference volume in-out) were performed Bilirubin and protein concentration of the outflowing fluid were also measured

The χ^2 method was applied for the calculation of difference between the mortality rates of the two groups To determine the significance of survival curves Wilcoxon s rank sum test (4) was used

RESULTS

The birthweights severity of HMD symptoms and blood pH values at admission were comparable in the two study groups (Table I) The survival curves of both groups during the first 5 days of life are given in Fig 2 More infants in the PD group survived the fifth day than in the control group 21 out of 50 infants and 7 out of 42 respectively

Clinical and laboratory observations PD was well tolerated by the premature infants and no serious complications were observed in particular respiratory embarrasment was not noted Besides the hindered outflow of the dialysis fluid (only by the intermittent method) transitory abdominal distension was observed in 4 cases In some cases oedema of the genitalia appeared but subsided soon after the end of the treatment

The first signs of clinical improvement were observed as early as 4 hours after PD was begun and marked improvement was usually present after 24 hours Acid base values were in most cases normalized within 12 hours The bodyweight of the infants fall only slightly but regularly during the treatment

The outflowing dialysis fluid was usually markedly yellow but its bilirubin content did not surpass 1 mg/100 ml The protein content of the fluid fluctuated within wide limits and the total protein loss amounted to 1 to 2 g daily

An autopsy was performed in each fatal case No signs of peritoneal inflammation were noted

Table 1 *Distribution and mortality rate according to prognostic factors in infants in the trial*

	Dialysed		Probability	Controls	
	No	Died	*P*	No	Died
Birthweight kg					
< 1 00	6	5	NS	6	5
1 00–1 50	19	13	NS	17	16
1 50–2 00	22	10	< 0 05	16	12
2 00 <	3	1	NS	3	3
Severity of HMD on admission according to Weisser s score					
0–I	7	1	< 0 05	10	6
II	26	14	< 0 01	24	21
III	17	14	NS	8	8
Blood pH on admission					
< 7 00	5	4	NS	3	3
7 00 7 20	20	14	< 0 05	19	18
7 20 <	25	11	NS	20	14
Total	50	29	< 0 02	42	35

[a] NS – not significant

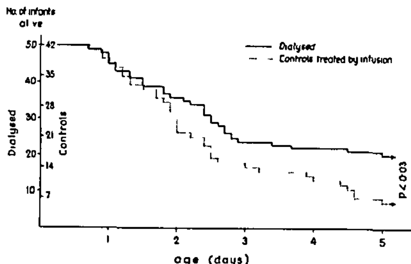


Fig 2 Survival curve in the 5 days of life of HMD of prematures treated by peritoneal dialysis and by alkali glucose treated controls

GENERAL CONCLUSION AND PLANNED FURTHER STUDIES

On the basis of our present clinical experimental series we may state that PD can be performed on premature infants without any remarkable difficulty The method of continuous dialysis does not appear to cause any serious stress to the infants

We are now striving to inciate PD when possible, at an earlier phase in HMD in premature infants [1] However, more studies are necessary to determine the value of PD in the treatment of HMD in premature infants Further clinical experiments are planned to investigate the therapeutic effects of PD by the control of arterial oxygen tension measurements

SUMMARY

Intermittent or continuous PD has been employed in the treatment of 50 selected cases of HMD of premature infants Twenty one infants treated by this method recovered whereas only 7 of 42 survived in a comparable control group treated with alkali and glucose

[1] Results of these studies *will be published in Acta Paediat Ac Sci Hung 11* No 3–4 1970

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CASE REPORT

TRISOMY 18 WITH OVARIAN DYSGENESIS

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Trisomy 18 was first described by Edwards et al in 1960 (3) It was then thought to be a rare syndrome but more than 200 cases have been published since New anomalies with varying frequency have been added to those initially described and the clinical picture has become more complex Furthermore cases with the clinical characteristics of trisomy 18 were found to have a normal karyotype (6 9) This has a special interest and has also been observed in other syndromes as in the male Turner syndrome

The purpose of this report is to present a case of trisomy 18 with ovarian dysgenesis and some additional uncommon pathological findings

CASE REPORT

Female infant born by normal delivery at Alexandra Maternity Hospital at 43 weeks of gestation Birth was uneventful She was the first child of a 25 years old epileptic mother who from the 5th month of gestation was on diphenylantoin sodium 100 mg and phenobarbitone 50 mg daily The father 31 years old, was healthy

The infant s birth weight was 1 980 g and the body length 46 cm She presented multiple congenital abnormalities (Figs. 1 2 3 and 4) (*a*) odd-shaped head with prominent occiput and narrow forehead (*b*) broad and flat bridged nose (*c*) low set ears with abnormal contours and a narrow external meatus (*d*) low hair line (*e*) small eyes (*f*) small mouth with hypoplastic mandible and high arched palate (*g*) upper extremities held at chest level with permanently clenched fingers and characteristic overlapping of the index over the middle and the small over the ring (*h*) hypoplastic thenar and hypothenar (*i*) small narrow pelvis (*j*) short webbed toes

On the second day of life she developed peri-oral cyanosis dyspnoea and many moist rales on both lungs The heart rate was 180 per minute Chest X ray revealed a globular enlarged heart. Her condition gradually deteriorated she developed generalized cyanosis apnoeic attacks and died at the age of 52 hours

Cytological Examinations

The chromosomes were studied in lymphocytes from cultures of peripheral venous blood Numerical analysis of 42 metaphases revealed 47 chromosomes in all the cells Structural analysis was done in 8 photographic karyotypes Length measurement and pairing of the chromosomes was easy The total length of the extra chromosome approximated the length of an 18 chromosome (Fig 5)

The sex chromatin was studied in amnion and buccal mucosa cells Out of 100 amnion cells 28 were found to be sex chromatin positive with one Barr body (the study of amnion cells was done because the present case was included in another research programme concerning all the newborn in the "Alexandra" Maternity hospital) Out of 200 buccal mucosa cells 22% were sex-chromatin positive with one Barr body

Dermatoglyphs

Prints were taken of the palms and fingers using the Walker (11) method but the examination was difficult as the fingers were abnormally flexed.

The most noticeable features were a failure of dermal ridge development on the thenar and hypothenar abnormal palmar creases on both palms and

This study was supported in part by Research Grants from the National Institute of Neurological Diseases and Blindness of the United States Public Health Service (no 5RO1 NBO-6390-07) and the Royal Research Foundation (no 767 and 849)



Fig 1 Patient's head—showing abnormal ears hypoplastic mandible small forehead

simian crease on the right palm All the finger tips had simple arches

Pathological Findings

In addition to the macroscopic anomalies previously described autopsy showed Atrioventricularis communis and patent ductus arteriosus Hypoplasia of the gall bladder Ovarian dysgenesis (Fig 6 *a*) Each ovary weighed 0 2 g and the histological picture was that of an undifferentiated gonad The cortex consisted of undifferentiated deeply stained epitheliallike germ cells which were arranged into partially anastomosed columns Scattered primordial follicles were found in a few places

DISCUSSION

The clinical features of the reported case especially those concerning the head and the extremities are characteristic of trisomy 18 as they have been previously described

Congenital heart disease is very common It was found in all the cases which had a postmortem examination (12) except one The usual type of abnormalities included ventricular septal defect patent foramen ovale and anomalies of the valves We did not find any reports of trisomy 18 with atrioventricularis communis, which was found in our case

Congenital abnormalities of the intestinal tract are frequent, but hypoplasia of the gall bladder is rare Warkany et al (12) in their review article mention only 2 cases of gall bladder hypoplasia

Ovarian abnormalities are also rare in trisomy 18 though the ovaries have not been studied thoroughly in all cases which underwent a postmortem examination The main abnormality was ovarian hypoplasia, the diagnosis being based on the size of the ovaries or on histological examination Weber et al (13) reported 1 case where the ovaries were small but there were no abnormal histological findings Butler et al (2) found 2 cases, among seven which were examined histologically, with a



Fig 2 Hand—showing typical position of fingers in the syndrome

marked reduction of the primordial follicles and marked proliferation of the granular cells forming small deeply stained irregular masses Lafourcade et al (7) reported a case with small ovaries but they did not describe the histological findings Warkany et al (12) reviewing the findings of 84 autopsies of trisomy 18 found 7 cases of ovarian hypoplasia but they do not say whether the diagnosis was based on the size of the ovaries or on histological findings Ovarian abnormalities were also reported in 2 cases of trisomy 18 combined with an XXX karyotype (10 8) and 1 case of XXX plus 18/XXX mosaicism (4) There was great reduction in the number of the primordial follicles with abnormal ovarian stroma In a fourth case of XXX 18 trisomy the ovaries were found macroscopically to be normal (5)

In our case the ovaries weighed one fourth of the normal weight for the neonatal period and the histological examination showed great reduction in the number of the primordial follicles Columns of epithelial like cells were also found These are considered as granular cells and as elements of the Pfluger columns from which the primordial follicles will be formed later It is impossible however to say with certainty whether these cellular elements belong to the initially appearing and consequently disappearing cortical columns from which seminiferous tubules are formed in the male (1)



Fig 3 Hand—showing hypoplasia of thenar and hypothenar



Fig 4 Feet—showing short webbed toes and widely spaced big toes

In cases if trisomy 18 with an XXX karyotype the ovarian abnormalities could be ascribed to the extra X chromosome (10) as similar histological abnormalities have often been found in cases with an XXX karyotype without trisomy 18 In our case no abnormality relating to X chromosome was found and therefore we believe that ovarian dysgenesis was another rare manifestation of the trisomy 18

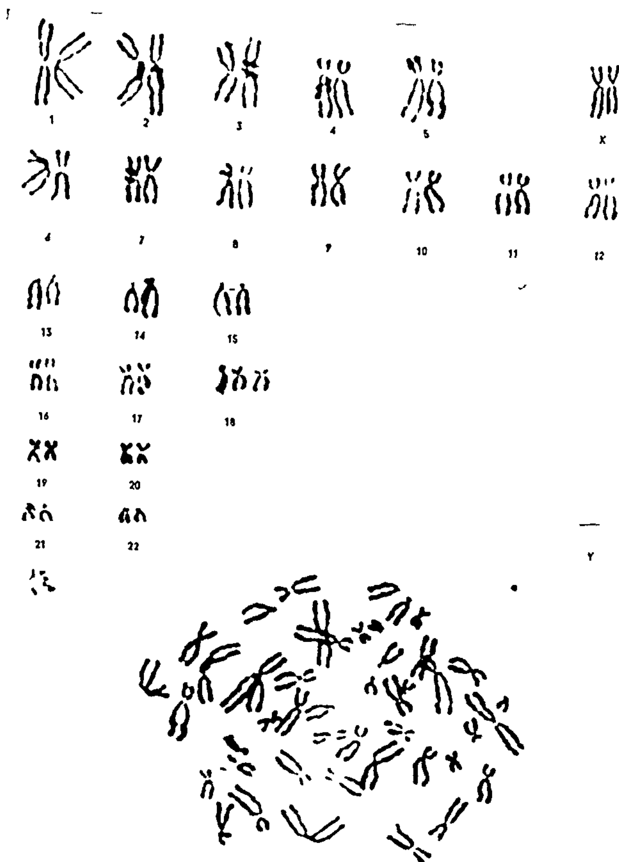


Fig 5 Karyotype of peripheral blood cells culture showing trisomy of chromosome 18 and XX complement (47 XX 18+)

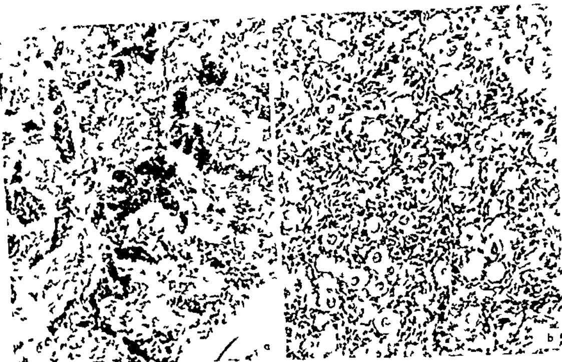


Fig 6 Ovary (a) of the patient showing deeply stained undifferentiated germ cells and (b) of a control case of the same age showing numerous primordial follicles Hematoxylin-eosin stain × 120

SUMMARY

A case of trisomy 18 with ovarian dysgenesis is described The chromosome study did not reveal any abnormality of the sex chromosomes and therefore we conclude that ovarian dysgenesis is another rare manifestation of trisomy 18

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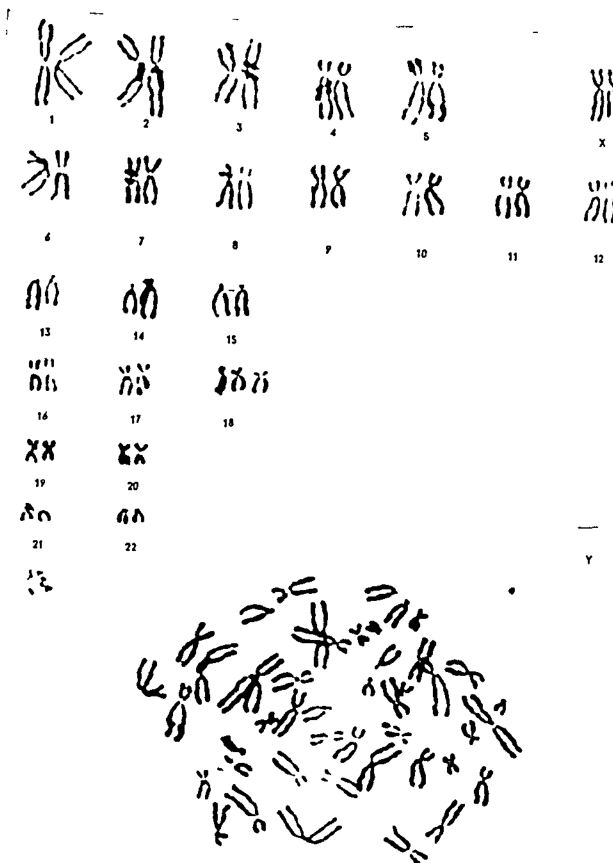


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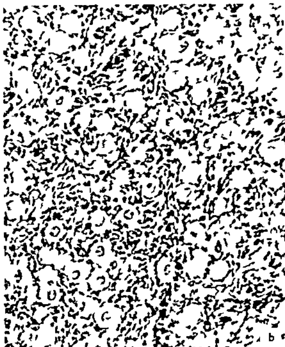


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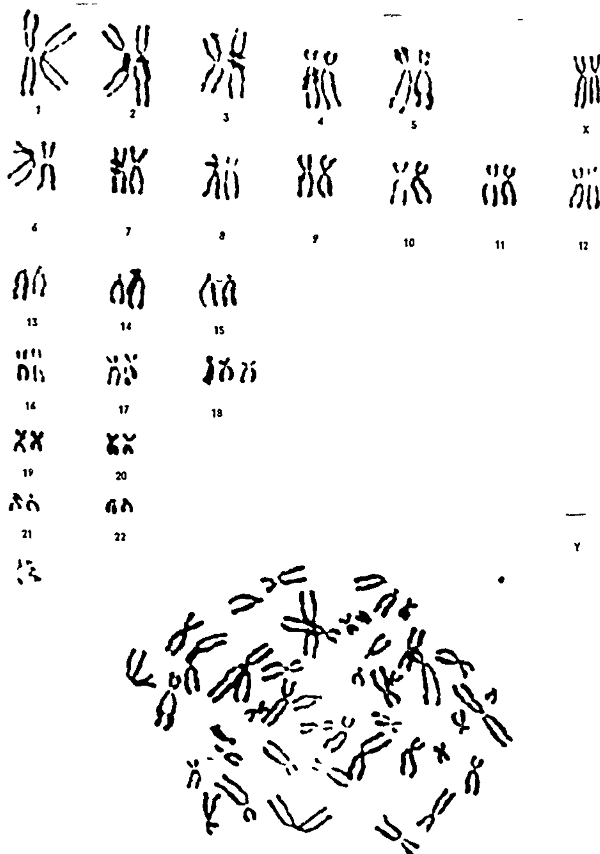


Fig 5 Karyotype of peripheral blood cells culture showing trisomy of chromosome 18 and XX complement (47 XX 18+)



Fig 1 Anne G when aged 3 months

variable abundance Few inclusions contain other more osmiophilic constituents (Figs 2 and 3)

DISCUSSION

There ar several reports in the literature con cerning infants under 6 months with gargoylism" (1–4 6 8–10) Typical features such as facial dysmorphism hepatosplenomegaly joint stiffness herniae and thickened gums have been observed at birth (1 6 10) Bone alterations also reported in the first days of life and charac terized mainly by a generalized rarefaction (1 6 10) become more typical as soon as 3–6 months (1–4 6 8 9) Corneal clouding was present at 2 and 3 months (3 4) Mucopoly saccharide excretion normal at birth (6 10) was noted to be increas d after a few weeks (2–4) and vacuolized lymphocytes have been observed at birth Enzyme and ultrastructural studies are scarcely available in these patients (3 10)

In our case several data support the diagnosis of mucopolysaccharidosis type I clinically there exist facial dysmorphism mental retardation hepatosplenomegaly inguinal hernia joints stiffness and corneal clouding biological studies show an increased mucopolysacchariduria affecting both heparan and dermatan sulfate and vacuolized lymphocytes However protruding sternum and high dorsal kyphosis are unusual findings Moreover the rapidly fatal evolution is a striking feature which so far has only been reported by Scott et al (10) and Iannaccone & Capotorti (6)

Analysis of acid hydrolases activities in the liver also yields an unusual result In mucopoly saccharidoses type I to III according to Van Hoof & Hers (12) the activity of β galactosidase is markedly decreased whereas in our case the activity of this enzyme remains within normal limits This latter fact may not be related to the early age of the infant since in Corbeel s patient the activity of β galactosidase was markedly decreased

Finally the ultrastructural aspect is not entirely similar to that observed in mucopoly saccharidoses type I to III In this group of diseases the different liver cell types contain large clear inclusions (3 5 7 11) In the pres ent case the inclusions are similar in content but they are much more numerous and generally smaller They often display evidence of coalescence possibly leading to the formation of larger vacuoles

SUMMARY

A mucopolysaccharidosis is diagnosed in an in fant aged 3 months Clinical and X ray data are not typical while biological studies reveal an increased mucopolysacchariduria affecting both heparan and dermatan sulfate Study of lysosomal hydrolases in the liver discloses a normal activity of β galactosidase Hepat c ultrastruc

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CASE REPORT

MUCOPOLYSACCHARIDOSIS IN A THREE MONTHS OLD INFANT

Clinical and Ultrastructural Studies

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CASE REPORT

Anne G a female infant was born on February 14 1968 after 8 months gestation She is the first child of a single woman aged 19 the father is unknown Delivery through breech presentation was uneventfull Birth weight was 2 450 g Postnatal clinical examination only reveals a very large fontanel Hypotony and hypoactivity are noted during the first 2 months of life and thoracic difformity at the seventh week When 2 months old the child is hospitalized for the first time for respiratory distress with cardiac failure Her height is at the 50th percentile (57 cm) whereas her weight is below the 10th percentile (3 490 g) Head circumference is at the 90th percentile (39 5 cm) and the fontanel is abnormally large (5 × 3 cm) Bulging forehead flattened nose with depressed nasal bridge as well as prognathism of the superior jaw give to the facies an obviously dysmorphic aspect (Fig 1) Palate is high arched There is a very important dorsal superior kypho scoliosis The thorax whose antero posterior diameter is markedly increased is deformed by the broad precordial arching There is no cardiac murmur A hepatosplenomegaly persisting after correction of the cardiac failure is noted Other anomalies include a moderate but generalized limitation of joint movements with thumbs kept in palmar flexion as well as a left inguinal hernia

X ray studies disclose a deformed thorax with a high dorsal kyphosis and a markedly protruding sternum Vertebral bodies show the oval aspect usual at this age Tubular bones do not reveal any alteration Electrocardiogram is normal Caryotype and chromatography of urinary aminoacids yield no abnormality Toxoplasmosis and rubella antibodies are absent Fundi and corneae are normal In peripheral blood 8 % of the lymphocytes display abnormal vacuoles containing dense central granules Bone marrow is normal Urinary excretion of mucopolysaccharides expressed in mg hexuronic acid is increased (21 5 mg per liter) Column chromatography performed according to a previously described technique (5) isolates important fractions corresponding respectively to heparan and dermatan sulfate Fragments of liver are obtained through puncture biopsy and submitted to enzyme and ultrastructural studies

When 3½ months old the child is discharged but rehospitalized 3 weeks later because of another episode of respiratory distress Height and weight curves show a marked inflexion Hepatosplenomegaly is more conspicuous Slit lamp examination of corneae reveals the presence of fine granular opacities Psychomotor development is considerably delayed and corresponds to an age of 1 month This delay affects more postural control than social reactions The child leaves the hospital when 5 months old and dies at home 1 month later after a third episode of acute respiratory distress

Enzyme studies

Activities of liver acid hydrolases were assayed by Van Hoof and Hers in the Laboratory of Physiological Chemistry of the University of Louvain The activity of β galactosidase is more than four times higher than the mean activity observed by these authors in a group of 14 patients belonging to the mucopolysaccharidoses type I to III (12)

Electron microscopy

In most hepatocytes and Kupffer cells, the cytoplasm is filled with large clear inclusions limited by a unit membrane Their main component is a moderately osmiophilic substance scattered as a fine protein like precipitate of

ture shows in both parenchymal and Kupffer cells the presence of clear inclusions analoguous to those found in typical mucopolysaccharidoses although more numerous and generally smaller

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Fig 2 Hepatocyte Large clear inclusions contain a moderately osmiophilic protein like precipitate coalescing sometimes in more dense areas *ve* small vesicles limited by a unit membrane *m* mitochondria *er* ergastoplasm × 22 000

Fig 3 Hepatocyte filled with smaller inclusions Some of them contain a few myelin like elements (*my*) and or osmiophilic granules (*arrows*) Coalescing figures of several inclusions are observed *g* α glycogen *en* dilated endoplasmic reticulum 000

Another infant who was in a respirator for a 24 hour period with anoxygen concentration of as much as 90% later developed a subdural hygroma which was excised Evidence is now present of delayed psychomotor development and non specific abnormality on the EEG

The third infant was delivered in the 33rd week of gestation and showed severe asphyxia During the first weeks of life this patient developed pneumothorax in conjunction with respirator treatment and had recurrent episodes of cardiac arrest Respirator treatment extended over a period of 4 months and the patient was extubated at 7 months There is evidence of significant respiratory involvement with tracheomalacia marked retractions and hyperinflation on roentgenograms of the chest The EEG shows non specific abnormality with left sided *temporal preponderance* However the psychic and psychomotoric development is normal

Bengt Hagberg & Leif Hambraeus *Atypical case of leucinosis (maple syrup urine disease)*

A 5 year-old boy with a clinical picture of dwarfism microcephaly with imbecillity severe motor retardation and congenital cataracts is described The patient was born 3 weeks before term and his birth weight was 1 860 g During the neonatal period he had a clinical history consistent with a prenatal brain damage and was in a bad condition with convulsions and abnormal neonatal neurology At repeated follow up examinations it was found that the boy s somatic and psychomotor development was markedly delayed

At repeated occasions this boy had very peculiar and unexplained episodes lasting 6–12 hours and consisting of vomiting a semicomatous state and sometimes also convulsions

During one of his episodes a biochemical study of the amino acid pattern in blood cerebrospinal fluid and urine revealed a marked increase in the concentrations of the branched amino acids valine leucine and isoleucine This pattern was most pronounced in the blood and cerebrospinal fluid In the urine abnormal concentration of ketoacids was demonstrated When the patient s episodes had stopped these abnormalities were normalized It is suggested that this patient represents a transient and atypical form of leucinosis (maple syrup urine disease)

Bengt Hagberg Hans Kollberg Patrick Sourander & Hans Olof Akesson *Infantile globoid cell leucodystrophy (Krabbe s disease)*

A series of 32 cases of globoid cell leucodystrophy was collected from a 15 year period and studied particularly from clinical and genetic aspects The series was considered representative of the real occurrence of the disease in Sweden during the period in question

The clinical picture and the course of the disease were usually surprisingly uniform from case to case The median age at onset was 4 months and at death 13 months The course of the disease was divided into three clinical stages the characteristics of which have been described in detail

Laboratory aids for diagnosis have been discussed on the basis of experiences from this series Histological changes for diagnosis were summarized from studies of biopsy specimens from peripheral nerves and brain

A geneto-statistical study showed that the probands with GLD did not differ from the standard population in either maternal age or birth rank that there was no apparent consanguinity in this series that the estimated incidence was approximately 1 9 × 10 and that the disease was diffusely spread over the country Finally from the family analysis it was concluded that globoid cell leucodystrophy is most likely inherited as an autosomal recessive disease

Acta Pædiat Scand 60 102–111, 1971

PROCEEDINGS OF PAEDIATRIC SOCIETIES

SWEDISH PAEDIATRIC SOCIETY

Meeting April 26, 1969

Lars Victorin, Anders Gustafson Ingemar Kjellmer & Ragnar Olegard *Intravenous administration of fat to premature infants*

Early administration of intravenous fluids and calories appears to reduce both the morbidity and mortality of prematurely born infants Administration of a 10% solution of invertose (65 ml/kg daily) is commonly used but probably only amounts to one third or one fifth of the actual requirement As a mean of increasing the caloric intake intravenous fat in the form of Intralipid Vitrum (0 5 g/kg in a single dose) was administered during a 5 min period This solution provides about 2 cal/ml, which is roughly four times more per volume unit than the above-mentioned invertose solution

This intravenous fat solution may be equivalent to endogenous chylomicrons It is metabolized in the same manner as orally administered fat apart from the fact that it lacks A protein which must be supplied by plasma if separation of triglycerides is going to occur

In 10 cases intravenously administered fat was rapidly metabolized and the initial hyperlipemia was in most cases no longer evident after 2 hours and had a half life of 20–30 min In a few cases the level of total lipids remained elevated for a longer period—i e between 6 and 30 hours These cases were further analyzed by paper electrophoresis and agarose electrophoresis It could be shown that this was not due to delayed handling of the chylomicron fraction in the liver The level of this fraction falls at the same rate as that seen in the other prematures The relatively high total lipid level between 2 and 30 hours of age was due to prebeta lipoproteins—in other words second generation lipoproteins formed in the liver

In all cases the blood sugar concentration was above the hypoglycemic limit (30 mg/100 ml) No pathological increase in ketone bodies was observed

Lars Victorin & Roy Cooke *Follow up of infants with the idiopathic respiratory distress syndrome (IRDS)*

During a period of eighteen months dating from January 1967 until June 1968 all surviving infants with the diagnosis of IRDS (hyaline membrane disease) have been re-examined They were between 8 and 24 months of age at the time of this study The severity of their illness was classified as mild in 4 cases moderate in 15 and severe in 7 Five of these 7 patients were treated in a respirator The examination included careful physical and neurological evaluation ophthalmological and audiological testing roentgenogram of the chest EEG as well as developmental evaluation at home using Lindstam's (1967) modification of Griffith s developmental scale No definite pathological finding was noted in 23 of the 26 cases

One patient who required respiratory treatment until the age of 6 weeks showed mild retractions with stridor In addition the roentgenogram of the lungs showed alternate regions of hyper- and hypo inflation

they are usually short and blind From a roent genological point of view it can then some times be impossible to distinguish between ul cus and fistulae

The roentgenographic appearance of local foci with submucosal hyperplasia resembles that of polyposis and is the basis of another type of cobble stone pattern which is less coarse than that referred to above

Segmental involvement with alternating healthy and diseased segments of the intestines is common Sometimes it is only the mesen teric side of an intestinal segment that is af fected and spastic In such cases the opposite healthy side will bulge like a diverticulum

The roentgenogram of granulomatous entero-colitis contains a number of inflammatory changes that are not characteristic of this disease alone But the combination and the site of the changes are usually characteristic enough to allow differentiation of the condition from other diseases

N O Berg. *Pathology*

In 1966 Ehrenpreis et al discussed 17 juvenile subjects with Crohn s disease In 13 of these surgical resection of diseased intestinal seg ments was performed Of our 13 subjects only one was operated upon In this the ileocaecal region presented the classical morphologic le sions with tuberculoid granulomas in the mu cosa and in the enlarged lymph nodes

Peroral suction biopsies of small and large intestine and rectal biopsies showed intestinal lesions in another 9 subjects Of 38 specimens all sectioned serially in its entirety no abnorm ity was seen in 19 Multiple biopsies (3–9) were performed in 6 subjects and single biopsies in three

Characteristic lesions such as tuberculoid granulomas were found in three subjects Brun nerian gland metaplasia, lymfoedema or single giants cells were seen in a few biopsies In six specimens from three different subjects a flat avillous mucosa was seen in roentgenologically changed intestinal segments The changes dif fered from those seen in coeliac disease in mainly two respects the changes were more granulomatous with more neutrophiles and they reached down to the submucosa Of special interest was the occurance in four subjects of small focal lesions in intestinal segments with out distinct roentgenological changes mostly in the upper part of jejunum The diagnostic and pathogenetic significance of these discrete lesions is not settled

B Cavell *Gastric emptying in infants*

A new method for studying gastric emptying of human milk in infants is described The method makes it possible to study the whole emptying pattern of one meal without the need of complete aspirations and refillings of the stomach The technique involves gastric intuba tion and iterated intragastric volume determina tions using polyethylene-glycol (PEG) as a marker

Known amounts of concentrated PEG solu tion are given via the tube at each volume determination and the resulting increase in PEG concentration of the gastric contents after mix ing is measured Knowing this concentration the intragastric volume can be calculated

The gastric emptying pattern in infants aged 2–6 weeks showed an initial rapid emptying phase followed by a slower phase with a con stant emptying rate of 0 4 ml per minute and lasting until the stomach was empty In some infants aged 2–3 months however an exponen tial emptying pattern with a constant of dec rement about 1 5–2 5% was found

The variations of gastric emptying pattern from day to day within individuals seemed to be rather small

N W Svenningsen A Dahlqvist & B Lind quist *HBABA index in neonatal icterus*

Introduction

B Lindquist The relationship between the serum bilirubin concentration and bilirubin en

Meeting Oct 10, 1969

REGIONAL ENTEROCOLITIS (CROHN'S DISEASE)

B Hansing & G Meeuwisse *Clinical signs*

From 1963 until October 1969 we have seen 13 new cases of regional enterocolitis at the Pædiatric Department, University of Lund The age distribution was 6–14 years with maximum 4 cases at 10 years There were 3 girls and 10 boys One of the patients has an elder sister with the disease During the same time we had 17 cases of ulcerative colitis (14 girls and 3 boys) The diagnosis has been established on the clinical picture in combination with distinguishing radiological features, in some cases supported by pathological studies on intestinal biopsies The primary symptoms were diarrhea (4 cases), abdominal pain (5), suspect appendicitis (1) erythema nodosum (1) anal fissure (1), elevated ESR and gammaglobulin (1) Moreover, we observed digital clubbing (2 cases), short stature (2) delayed puberty (1) oedema (1) loss of weight (13) fever (6) All patients were anaemic with sideropenia and in most cases low TIBC 6/8 had subnormal levels of folic acid/s 8/8 had normal B_{12}/s 12/13 had elevated ESR Albumin/s was generally low Gammaglobulin/s was 2 10 in one case and above 1 20 g/100 ml in 6 other patients 3/3 had elevated ASTA 3/11 had a pathological xylostest RISA test showed in 2/2 cases proteinlosses in faeces more than 30 times the normal

In 3 cases there had been difficulties before establishing the correct diagnosis One of these had at another hospital passed a thorough endocrinological examination because of short stature, another was retarded in his growth had oedema but normal ESR, the third had elevated ESR and gammaglobulin but no symptoms directly referable to intestinal disease

All patients were treated with salazopyrin One had a stop medication after 3 weeks because of exanthema Another got his treatment only for one week and was then referred to the surgical department The rest have been treated more or less continuosly even during symptomfree periods The only surgically treated patient has just relapsed, the other patients are now in a good general condition In 5 cases we have added steroids in general low doses during short periods Especially in the 2 cases with proteinloosing enteropathy we have seen a very good response to this medication Salazopyrin alone did not improve these patients In 2 cases with intermittent salazopyrin treatment we could see a prompt effect of salazopyrin with rapid increase in weight and cessation of symptoms

W Mortensson *X ray picture*

Inflammatory lesions of the intestinal wall and mesenterium produce a widely varying picture as may be seen in roentgenograms of patients with granulomatous enterocolitis The lesions are situated mainly in the small intestine They are of essentially the same appearance in the large and in the small intestine The thickening of the intestinal wall causes narrowings of the lumen and the valvulae conniventes become irregular blunt and ill-defined Spasms due to the inflammatory changes also affect the width of the lumen in the acute stage of the disease In later stages cicatrization may cause permanent strictures Intestinal dilatation is rare, but may occur orally to the stenosed segment

Inflammatory thickening of the mesenterium and of the wall of the small intestine deforms the otherwise smooth curves of the loops the loops are separated from one another and assume a rigid course

The ulcerations usually extend longitudinally in the mesenterial side of the intestine and are often connected with one another by transverse ulcers with the formation of a cobble stone pattern The ulcerations may also be deep and perpendicular of diffuse and simulate the absence of mucosa

Fistulae may communicate with other hollow organs or with the surface of the body But

they are usually short and blind From a roentgenological point of view it can then sometimes be impossible to distinguish between ulcus and fistulae

The roentgenographic appearance of local foci with submucosal hyperplasia resembles that of polyposis and is the basis of another type of cobble stone pattern which is less coarse than that referred to above

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N W Svenningsen A Dahlqvist & B Lindquist *HBABA index in neonatal icterus*

Introduction

B Lindquist The relationship between the serum bilirubin concentration and bilirubin en

cephalopathy has been well established in Rh-hemolytic disease In nonhemolytic hyperbilirubinemia this relationship is, however, less well defined This is especially relevant for premature infants It is now well known that there are many factors contributing to the pathogenesis of bilirubin encephalopathy rate of hemolysis development of the enzyme systems, acid-base balance, presence of hypoxia and respiratory distress, administration of certain drugs, etc Since 1959 Odell and co-workers have in several reports pointed out that the relationship between the concentration of serum albumin and serum bilirubin is a better criterion than the bilirubin concentration alone in judging the risk for bilirubin encephalopathy This concept is based on experimental studies with ^{14}C-bilirubin, the concentration of ^{14}C-bilirubin in the central nervous system was correlated to the amount of free bilirubin (bilirubin not bound to albumin) rather than to the total bilirubin level in serum Bilirubin encephalopathy will arise when the serum albumin has an insufficient amount of binding sites for bilirubin

By *in vitro* studies the amount of bilirubin that can be bound to a certain amount of albumin can be determined and thus the relative amounts of free and bound bilirubin in a mixture of bilirubin and albumin can be calculated The situation is, however, more complicated in vivo the binding capacity varies i a due to presence of exogeneous and endogeneous substances which compete with bilirubin for the binding sites of the serum albumin Therefore it has been necessary to work out special methods to determine the so called reserve albumin (the reserve capacity of serum albumin to bind unconjugated bilirubin) Knowledge of the amount of reserve albumin in the single patient will probably give a more accurate background for evaluation of the risk for bilirubin encephalopathy than does information about the bilirubin level alone

Method

A Dahlqvist The measurement of reserve albumin is based on the ability of albumin to bind a suitable dye indicator This dye must be bound to albumin with a lower affinity than is the bilirubin, else the dye will replace bilirubin and the total albumin rather than the free (reserve) albumin will be measured A method utilizing PSP (phenolsulfonphthalein) was described by Waters & Porter in 1961

This method had the drawbacks of being laborious and time consuming and the amount of serum needed is fairly large A better method was described by Porter & Waters in 1966, utilizing HBABA (2-(4'-hydroxybenzeneazo) benzoic acid) and this is the method we have used The colour of the HBABA albumin complex is different from that of free HBABA, and therefore, if the measurements are made at suitable wavelength, the amount of HBABA albumin complex can be measured without separating it from the free dye This method is rapid (it is performed in a few minutes) and the amount of serum needed is small (0 2 ml, including the amount used for blank correction) In our experience it is accurate and reproducible The standard deviation was ±2 9% of the mean value when calculated from 30 assays of the same sample (each assay performed on a separate day)

The results of HBABA-binding assay are expressed as an index value where index 100 refers to a standard solution containing 4 g albumin per 100 ml The normal range is an index value of 70–100, and a value below 25–30 is supposed to indicate a risk for the development of bilirubin encephalopathy In a number of samples from patients with different degrees of icterus we compared the experimentally obtained value for the HBABA index with a theoretical value calculated from the assay of total albumin and bilirubin respectively The albumin concentration was determined by quantitative electrophoresis In a few cases the assay of albumin was also performed with high voltage immunoelectrophoresis (these assays were performed by C B Laurell Malmo) The theoretical HBABA index was calculated with the assumption that 1 gram of albumin can bind 17 mg of bilirubin which is the relation

Table 1 *Infants with bilirubin encephalopathy—clinical signs or verified kernicterus at autopsy (ki)—at HBABA index levels above or below 30 in group II*

HBABA index	Total no	Neonatal deaths		Surviving infants with bilirubin encephalopathy	
		Total no	No with ki	Neonatal signs	Late cerebral sequelae
>30	138	11	1	1	—
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found in test tube experiments The experimentally obtained HBABA index was in these sera up to 60 % lower than the theoretical HBABA index Only in a few cases did both methods give the same index figure This is in good agreement with the concept that in addition to bilirubin some other substances (endogenous and exogenous) are bound to albumin and that therefore the dye binding capacity of the serum should be a better measurement of the amount of reserve albumin available than are separate measurements of bilirubin and total albumin

Clinical investigation

N B Svenningsen The clinical investigation comprises 3 groups of neonates 0 to 7 days of age *Group I* 46 infants—22 preterm and low birth weight infants (LBW) and 24 fullterm infants (FT)—with a maximal serum bilirubin of 6.2 mg/100 ml during the neonatal period A significantly lower HBABA index on days 1 to 4 was found among the nonicteric LBW infants

Group II 175 infants—86 LBW—and 89 FT infants—with hemolytic (50) and nonhemolytic (125) hyperbilirubinemia Serum bilirubin and HBABA index were followed concomitantly in the neonatal period A significantly lower HBABA index among the LBW infants was demonstrated even in this group

A higher incidence of bilirubin encephalopathy was demonstrated among infants with low HBABA index (group II) irrespectively of the serum bilirubin level (Table 1) Eight out of the 11 (4+5+2) infants with low HBABA index and symptoms of bilirubin encephalopathy or kernicterus verified at autopsy were LBW infants Maximal serum bilirubin values between 12.0 and 17.8 mg/100 ml were registered in the cases with kernicterus In comparison to this no kernicterus was found at autopsy of another 9 infants with the same serum bilirubin level the same gestational age and birth weight but with HBABA index in the normal range above 30 The indications for exchange transfusion in this group were the empirically accepted serum bilirubin levels considered at risk

Group III 99 infants—46 LBW—and 53 FT infants—with hemolytic (28) and nonhemolytic (71) hyperbilirubinemia These infants were treated irrespectively of the serum bilirubin level with albumin infusion and exchange transfusion when HBABA index decreased rapidly towards or below 30 No kernicterus has been found among 7 infants dying in the neonatal period although HBABA index was below 30 in 3 infants Neither have any late cerebral sequelae been registered among the survivors The mean follow up period is now 11 months When albumin infusion (1 g/kg/day) was given a mean rise of serum bilirubin of 3 to 5 mg/100 ml was found within 6 to 8 hours This is probably the result of a bilirubin shift from extra to intravascular compartment

In a separate series of 52 infants with nonhemolytic hyperbilirubinemia the effect of albumin enrichment of donor blood (50 ml 20% human albumin per 350 ml donor blood) was studied Albumin enrichment was performed

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In a separate series of 52 infants with non hemolytic hyperbilirubinemia the effect of albumin enrichment of donor blood (50 ml 20 human albumin per 350 ml donor blood) was studied Albumin enrichment was performed

at every second exchange transfusion in this series A significantly higher and persisting rise of the HBABA-index was demonstrated in the albumin enriched cases

In summary the results of this investigation have demonstrated that the HBABA-index is a most useful and reliable test for evaluating the reservbinding capacity of the serum albumin A good correlation between low HBABA-index and the incidence of bilirubin encephalopathy was found even at serum bilirubin levels below the empirical levels for exchange transfusion Albumin infusion resulted in a significant rise of HBABA index As a consequence of these findings it is considered that exchange transfusion should be performed in some infants with relatively low serum bilirubin level, but at the same time also low HBABA index On the other hand exchange transfusion should not necessarily be performed in other infants with serum bilirubin above the conventional levels for exchange transfusion if the HBABA-index level is high

G Engleson, S Aronson & J Thorell *The Prader test and plasma HGH in children with short stature*

Short time metabolic test with exogeneous human growth hormone, HGH, the Prader test has been used by us as a diagnostic measure in 4 patients (*Arch Dis Childh 39* 535, 1964) The test was performed as a balance study The protein and caloric intake of the patient was fixed The nitrogen excretion in the urine was estimated from Urea-N + Ammonium N The patient was given HGH (by courtesy received from M S Raben) and the percentual decrease of the urinary N was computed The plasma levels of HGH at the intravenous insulin test and arginine load were compared with the findings of the Prader tests Two patients had maximal values of plasma HGH less than 6 ng/ml These children retained pathological amounts of nitrogen, 43 and 44% respectively at the Prader test One patient who had normal plasma HGH values showed no N retention The fourth child had intermediate values in plasma HGH Her maximal value at two insulin tests was 11 ng/ml Her N retention was slightly elevated, 21%

The diagnostic value of the Prader test has been questioned So far our data show a good correspondence between the results of this test and plasma HGH values

Lately another child with short stature has been found to have plasma HGH less than 6 ng/ml He also had an increased N retention of 36% at the Prader test

N-R Lundstrom *Ultrasoundcardiography in infancy and childhood*

Ultrasoundcardiography has been performed on more than 200 children The age of the children has varied between 1 day and 16 years Most of the investigations have been performed without premedication and all without discomfort for the patient

The method has been found valuable for demonstration of dilatation of the right ventricle both in cyanotic and acyanotic congenital heart disease In the study of the tricuspid valve we have found pathologic findings in tricuspid atresia and Ebsteins anomaly

The method has been found valuable in evaluation of the state of the mitral valve Pathological findings have been obtained in congenital mitral stenosis mitral atresia and other forms of hypoplastic left heart syndrome cleft mitral leaflet in endocardial cushion defects and other forms of mitral regurgitation

We have also studied the outflow tract of the left ventricle Pathological findings have been obtained in cases with membraneous subvalvular aortic stenosis and hypertrophoc obstructive cardiomyopathy

Even in infants and children the method has been useful for demonstration of pericardial effusion

Hans Ahlstrom *External detection of ¹³¹I Hippuran in the diagnosis of left to right shunts*

Cardiac left to-right shunts can be discovered by external detection over the lung field after intravenous injection of a radioactive isotope In 35 patients (adults and children) ¹³¹I Hippuran was injected during cardiac catheteriza tion and a scintillation detector was placed over an apical lung field The peak activity (C_1) and the activity after an interval equivalent to the build up time (C) were measured Ox ygen saturation data were utilized for quantita tion of the magnitude of the shunts These results were compared with the ratio of activity C to C_1 expressed as a percentage

In 15 patients without left to-right shunts C /C_1 were less than 50 ′ In 5 patients with small shunts (lung flow/systematic flow less than 2/1) C_2/C_1 were 50–65 % In patients with large shunts (2/1 or more) the ratio were more than 65 ′

The method is simple and should be valuable in children and it seems to be a reliable method in screening patients with heart murmurs of un certain etiology

R Stensman & B Ursing *Epilepsia termobalnearis—an unusual case of reflex epilepsia*

A case of reflex epilepsy in an otherwise healthy 5 year-old boy was presented and brief ly discussed When the patient was taking a hot bath he had attacks of focal temporal type and simultaneously left temporal abnormality appeared in the otherwise normal EEG The precipitating stimulus was found to be specific Water with low temperature hot air among other tests did not provoke seizures or EEG abnormalities Antiepileptic treatment with car bamazepine (Tegretol®) has so far been suc cessful

Lars Holmberg *Solitary mastocytomas*

Solitary mastocytoma is a form of mastocytosis with only one isolated skin lesion Eight cases of solitary mastocytoma among children born in 1966–68 in Malmo have been recorded Six of them have been submitted to close investiga tion In most cases the tumour appeared at birth or soon after The diagnosis was con firmed by histological examination No signs of systemic mastocytosis were revealed by X-ray survey of the skeleton or by bone marrow studies

One patient had an attack of generalized flushing and another had an attack of bronchial asthma on mechanical stimulation of the tu mour In the first case there was an increased urinary excretion of histamine while it was normal in all the others The urinary excretion of 5 HIAA was normal The coagulation mech anism was normal and in particular no patho logical amounts of a circulating anticoagulant of the heparin type were demonstrated Further more there was no local fibrinolytic activity in the tumour itself

Inger Magnusson & Sven Ake Kornfalt *Wilms's tumour*

The material was collected from the Depart ments of Radiotherapy and Paediatric Surgery University Hospital Lund It covers a 15 year period (1953–1968)

From 1958 to 1963 the number of new cases diagnosed each year in Sweden varied be tween 11 and 19

The present material consisted of 33 chil dren (15 boys and 18 girls) The tumour was bilateral in 5 left sided in 17 and right sided in 11

One patient received no treatment The re maining 32 were distributed among three groups according to treatment

Group 1 (3 patients) Only excision of the tumour No survivors

Group 2 (6 patients) Preoperative radio-therapy followed by excision of the tumour and postoperative radiotherapy One survivor

Group 3 (23 patients) Immediate nephrec-tomy followed by postoperative radiotherapy

Nine survivors Of the survivors, 6 were followed up for more than 5 years 1 for 5 years, 1 for 2 years and 1 for less than 2 years Five of the 9 survivors received orthovoltage X-ray (170 kV, 1 0 mm Cu HVL) and the remaining four ^{60}Co irradiation

The initial symptoms were abdominal swelling or a palpable mass in 26 cases, pain in 12, nausea and vomiting in 9 and hematuria in 6

Of the 32 patients treated 10 have survived and nine of them were followed up for more than 2 years and showed no signs of recurrence when last seen Eight of them belonged to group 3 (nephrectomy followed by postoperative radiotherapy) Immediate nephrectomy plus simultaneous administration of Actinomycin D followed by postoperative radiation is recommended

The importance of early diagnosis and centralization of treatment and close cooperation between the paediatric surgeon and the radiotherapist is stressed

B Lindquist & G Meeuwisse *Should gastroenteritis in infancy be treated with antibiotics?*

Starting with a case history of a child who—because of an ordinary enteritis—within a few days was given different antibiotics but no dietary prescription, the draw backs of routine treatment of gastroenteritis with antibiotics were discussed Some recently published articles propagating the routine usage of Neomycin in children below 2 years of age hospitalized for gastroenteritis demonstrate that the disadvantages of such and similar practice are not always given due consideration Slowly absorbable antibiotics like Neomycin may cause parenteral and enteral side-effects Parenteral side effects are the consequence of absorption to an unforeseen degree Risk of an enhanced absorption exists in the neonatal period but also later on in life if the intestinal tract is subjected to inflammatory changes By this mechanism Neomycin may cause deafness and renal damage The enternal side effects consist of 1) disturbed intestinal micro-flora, including the risk of dominating growth of e g *Staphylococcus aureus* or *Candida albicans* 2) increased transfer of resistancy factors between strains and sorts of bacteriae In this way bacteriae (e g *Proteus*) with multiple resistancy will arise and they will constitute a potential risk for the environment of the patients As in countries like Sweden acute gastro-enteritis is rarely caused by bacteriae the use of antibiotics is not indicated unless we are dealing with a documented epidemic with a pathogenetic *E coli* strain Moreover, routine administration of antibiotics may lead to diminished interest for dietary treatment and ignorance of instructions for barrier ward which are issued to prevent spread of diarrhoic disorders within the hospital

Jan Lagergren & Richard Stensman *EEG and the clinical course after cerebral hypoxia in childhood*

An almost 3 year-old boy was deeply unconscious after an accident for about 24 hours and regained consciousness gradually Two weeks after the accident he could sit up without support and started to talk again After 4 weeks he could walk unsupported In spite of the good and continuous clinical improvement there was in the EEGs an increase slowing with maximum on the 17th day after the anoxia It was not until two months after the accident that the EEG was normal

The other boy was 12 years old when he by accident was strangulated He was unconscious for about 15 hours From the second day after the accident he was active concentrated and physical examinations showed normal motor and neurological findings An initial EEG showed an increased amount of slow activity bilaterally After a slight improvement there was—in spite of the clinically excellent condition of the patient—a deterioration of the EEG with a maximum on the 6th and 8th day The

increased amount of slow activity was then decreased again and the EEG was quite normal one month after the accident

In our 2 cases the discrepancy between the clinical improvement and the late changes in the EEGs is interesting. Delayed neurological deterioration following anoxia has attracted little attention in the literature. In 2 patients dying from brain damage after anoxia an extensive demyelinisation was found. The background to the late EEG changes in our cases may be of similar nature.

We think more intense investigations with serial EEGs are of great interest in cases with cerebral hypoxia and necessary for evaluation of the prognosis.

R. Lagercrantz

PROCEEDINGS OF PAEDIATRIC SOCIETIES

THE FINNISH PAEDIATRIC SOCIETY

Meeting Febr 14 1970

J R Bierich (Tubingen West Deutschland) *Etiopathology and modern treatment of sexual precocity*

Meeting Apr 24, 1970

Katri Malmivaara *Psychiatric consultations in paediatric hospital wards*

Experience derived from psychiatric consultations on paediatric wards at Aurora Hospital, a general city hospital in Helsinki, was presented There has been a small psychiatric unit at this hospital for 18 years Regular psychiatric consultations have been available for paediatric patients since 1957 These include case conferences together with paediatricians psychiatric examinations different kinds of therapy (individual psychotherapy for children and parents, group psychotherapy family therapy) and *supervision of medical staff on children s wards* An analysis of the obstacles and achievements in this work was presented

Gunvor Vuoristo *Brief Psychotherapy*

For a paediatrician in daily practice it is possible to prevent neurosis, if he is interested in psychotherapy When child psychiatric consultation is available there may still be difficulties in communication due to differences in the quality of language of a pediatrician and a child psychiatrist Another difficulty turns up when a paediatrician finds out that the patient needs psychotherapy and thus disrupts his ordinary time scedule Even if it would be possible to increase the amount of training in psychology, sociology and childpsychiatry, a keen interest in the developing personality of a growing child is required in order to become a successful psychotherapist

Juhani Lappalainen

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ANNOUNCEMENTS

SECOND INTERNATIONAL SYMPOSIUM OF PAEDIATRIC NEPHROLOGY

The 2nd International Symposium of Paediatric Nephrology will be held in Paris from 25–28 August 19 1

Pr sid nt P Royer

General Secretaries R Habib and H Mathieu

The scientific programme will deal with the follow in topics (1) Developmental nephrology (2) Im munolo ical aspects of Paediatric Nephrology (3) Chronic uremia and its treatment (4) Free com munications

The languages of the symposium are Enelish French and Spanish. Attendance will be limited to 350 participants Active inve tigators in the field in terested by the Symposium are requested to inform as soon as possible Dr Renee Habib H spital des Enfants Malades 149 rue de Sevres Paris 15 France

ASSOCIATION FOR PAEDIATRIC EDUCATION IN EUROPE

After various surveys discussions and seminars on paedia ric edu ation in Europe sponsored by the European Office of the World Health Organization it was felt that the time has come for the founda tion of an official Association the main purpose of whi h will be the paediatric education at all levels in th countries of the European region Numerous informal meetings and discussions took place until on September 29th 1970 in Athens the new Asso ciation was officially founded It is called Associa tion for Paediatric Education in Europe (A P E E) and the aims are

a) To encourage improvements in paediatric educa tion
b) To collect exchange and disseminate informa tion about paediatric education
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The first Executive Committee was elected consist ing of the following G M H Veeneklaas Leiden President S A Doxiadis Athens Secretary Treasurer J Houstek Prague Nathalie Masse Paris S Sjolin Uppsala

The next meeting of the Association will take place in Vienna before the 13th International Con gress of Paediatrics The address of the Secretary is Institute of Child Health Athens 608 Greece

EUROPEAN STUDY GROUP ON CHILD NEUROLOGY

Under the sponsorship of the Spastics Society of En land and arranged by it Medical and Educational Unit directed by Dr R Ma Keith, a meeting of 57 specialists in child neurology was held in Oxford during the last week of September 1970 for discus sions on future international communication It was considered that much could be gained by following the pattern of meetings which have taken place in Oxford for more than 1? years by courtesy of the Spastics Society of England The value of such meet ings has lain in the fact that they have been limited to 50–60 participants who have given papers and have profited from free and extensive di cussions It was decided therefore to organize a "European Study Group on Chi d Neurology This would con vene every third year and would comprise a perma nent body of 30–40 members and in addition parti cipants selected for each coming meeting by a council according to the subjects to be discussed Such parti cipants might very well be experts from other fields than pure clinical child neurology The total number of persons taking part would be limited to 60

A preliminary council of 6 members was elected representing different regions of Europe namely Dr Neil Gordon (United Kingdom and Ireland) Dr Gilles Lyon (France Italy The Peninsula) Dr Jan Willemse (Benelux) Dr Franz Schulte (Central Europe) Dr Ivan Lesny (East Europe) and Dr Bengt Hagberg (Scandinavia) The council elected Dr Hag berg as its secretary and Dr Sven Brandt was ap pointed chairman of the first meeting to be held in Scandinavia in the autumn of 1973

Sven Brandt

aspects of the progressive brain disorders would have been quite an advantage

The book presents itself as a nice printing but it is unavoidable that there are printing errors hopefully only few Many of the figures are of high quality

The part on psychology and psychiatry offers much interesting reading and includes some remarks on children in hospital child-doctors relation etc. which is rare and valuable to find in such a work

This book is of value to a complete library both for readers with a special interest in neuropaediatrics and related areas as well as for persons interested in e g infections of the CNS and many other problems

Personally I believe that in order to keep the reader up-to date smaller books on single parts of this enormous subject of neurology and psychiatry are of greater help to the daily work but still giants of the *present type offer much information to all of us*

J C Melchior

G Joppich & H Wolf (eds) *Metabolism of the new born child* Hippokrates Verlag Stuttgart 1969 448 pp illus DM 75 —

Some European scientists convened for a symposium on the Metabolism of the Newborn Infant in Deides heim Germany in October 1968 The present book is a wellwritten and most useful report from this meeting The editors have had the initiative and courage to publish the different sections partly in German partly in English all depending upon the preference of the author

All the authors are established specialists and their combination of presenting new data and the main thoughts in the field at the same time is advantageously done The chapters cover relationship between fetal and maternal metabolism the metabolism of amino acids proteins fat and carbohydrates the enzymatic and hormonal control of metabolism as well as a short section of inborn errors of disease

Gosta Rooth

G Henneberg (ed) Masernschutzimpfung Abhandlung a d Bundesgesundheitsamt Heft 8 Springer Verlag Berlin Heidelberg and New York 1969 91 pp DM 22 —

In USA vaccination with attenuated live measles virus vaccine has been practiced on a big scale for many years It has resulted in a marked reduction in morbidity and an apparently stable immunity Side effects and severe complications have been rare. These American experience are incompletely reviewed in Masernschutzimpfung" In this book massprophylaxis against measles is not recommended Inactivated measles vaccines which give less stable immunity and in some cases atypical severe reactions to live (wild or attenuated) virus is favored In USA this type of vaccine is not recommended any more

The current problem about the "slow virus infection possibly resulting in progressive sclerosin encephalitis is not touched upon This book covers the subject incompletely which is especially noticeable for an official document

Rutger Lagercrantz

P Krepler *Grundlagen und Fortschritte der Leukamiebehandlung beim Kinde* Beihefte zum Archiv fur Kinderheilkunde No 62 Ferdinand Enke Verlag Stuttgart 1970 158 pp illus. 18s DM 32.

During the last decade such a large number of investigations concerning childhood leukaemia have been published that it is almost impossible for the ordinary pediatrician to keep up with the literature in this field The book of Krepler gives a good review of the present situation of childhood leukaemia

Until 1963 the median life expectancy for patients with acute lymphatic leukaemia was about 10 months. This was the result of prednisone and purinethol therapy and folic acid antagonists administrated orally Presently we possess about 10 drugs effective against acute lymphatic leukaemia There is however clinical experience only with a few combinations of these drugs The best centers have now reached a median life expectancy exceeding 30 months

A special chapter is dealing with immune therapy of leukaemia Although there are great practical problems in this field definitely positive results have already been reached among others by Mathe and his associates in France The immunotherapy seems to increase the number of cases with a definite cure of leukaemia There is still much research to be done and new *therapeutic regimens are in progress* The review by Krepler which includes a large number of references possesses a great value for those who treat children with leukaemia and for investigators in this field as well

Eric Ivar Wallgren

Acta Pædiat Scand 60 115 1971

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The Editorial Board of *Acta Paediatrica Scandinavica* wishes to express its sincere gratitude to the following persons outside the Advisory Board who have acted as referees during the past year The standard of the journal depends to a very large extent on the skill and interest of these reviewers

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ULTRASOUNDCARDIOGRAPHY IN INFANTS AND CHILDREN

NILS RUNE LUNDSTROM and INGE EDLER

From the Departments of Paediatrics and Cardiology
University of Lund Lund Sweden

Ultrasoundcardiography (UCG) was introduced by Edler & Hertz in 1954 (6) Since then the most important application of UCG has been the study of the movements of the anterior mitral leaflet for diagnosis and evaluation of the degree of mitral stenosis (5 7 12) The method has also been found useful to demonstrate the presence of pericardial effusion (2 10 16 19) left atrial thrombosis (2) and tumours in the left atrium (8) The possibility of studying the movements of the anterior tricuspid valve in certain cases has also been reported (1 3 8 15) Preliminary results of UCG in congenital heart disease with left to right shunting were presented by Jacobi *et al* (14) Ultan et al (26) have more recently presented preliminary results of ultrasoundcardiography in congenital heart disease with particular reference to those with left to right shunting but also in cases with subaortic stenosis Six children were included in this study but otherwise we have been unable to find publications on ultrasoundcardiography in infants and children A systematic study of ultrasoundcardiography in infants and children particularly in cases of congenital heart disease has been commenced and we will present part of our results

MATERIAL

The material consists of children without heart disease and children with various forms of heart disease All age groups below 16 years of age (Table 1) are represented The children without heart disease were admitted to hospital for other causes than diseases affecting the heart or circulatory system In these children the auscultatory findings of the heart and the electrocardiogram were normal The children with heart disease were usually investigated with heart catheterization and angiocardiography during their stay in hospital About one quarter of the material is comprised of infants below 1 year of age the youngest being only a few hours old

METHOD

Ultrasound examinations were carried out using an ultrasonoscope Smith Kline Eskoline 20 This instrument produces 1 000 pulses/sec and utilizes a 2 25 megacycle transducer of 0 75 inch diameter The transducer acts as a sound transmitter for 1 μsec and as a sound receiver for the remaining 999 μsec until the subsequent pulse The transducer is connected to a cathode ray tube At each pulse the electron beam starts at the left side of the screen and moves to the right along the *x* axis at a constant speed Immediately after emission of each pulse the transducer becomes inactive and is mechanically excited by the returning echoes These echoes are converted into an electrical signal which is then amplified and fed to the y plates of the cathode ray tube This results in a vertical deflection of the electron beam The echo-signals are thus seen as vertical deflections on the oscilloscope screen (Fig 1 A) The horizontal distance between the left border of the screen and the echo-signals is a measure of the time necessary for the pulse to travel from the transducer to a reflecting surface and back again As the pulse rate from the transducer is 1 000 pulses/sec the distance between the transducer and the reflecting surface is measured 1 000 times/sec

As long as the velocity of the sound is the same in all the media it transverses the horizontal distance between the vertical oscillations on the screen will be a measure of the distance between different reflecting surfaces If the echo-giving structure moves towards the transducer the echo-signal travels along the horizontal axis towards the left hand side of the

Table 1 *Survey of material*

Diagnosis	Age	No of patients
No heart disease	1 week–15 years	45
Congenital heart disease	1 day–16 years	224
Primary myocardial disease	2 weeks–15 years	12
Pleural effusion	5 months–15 years	4
Right atrial thrombosis	1 year	1
Total		286

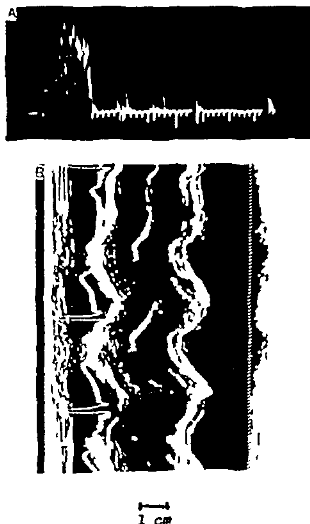


Fig 1 (A) Polaroid photographic picture of the oscilloscope screen as it is seen during scanning The transducer was directed towards the aortic root (B) Photographic picture of one vertical sweep of the signals from Fig 1 A after the signals were converted into bright spots An electrocardiogram was incorporated in the registration If this picture is turned 90 clockwise the presentation is the same as in the following figures

screen Alternatively if the echo-giving structure moves away from the transducer the echo-signal moves to the right hand side of the screen

The movements of the signals cannot be directly recorded on film but are valuable for scanning the field and for choosing a desired echo In order to record on a film the echo signals are transformed into bright spots along the invisible baseline The strength of the echo is represented in this case by the brightness of the spot The invisible baseline with the spots is swept upwards vertically on the screen Using a Polaroid camera a photographic picture of the screen during one full sweep shows all horizontal movements in wave form (Fig 1 B) A single lead electrocardiogram is incorporated in the recording To facilitate measurements a time marker and calibration marks representing each centimeter are recorded

Further by using a dual beam oscillograph with a four trace amplifier on one of the beams upto four different heart parameters such as electrocardiogram and phonocardiogram can be recorded simultaneously while the ultrasoundcardiogram is recorded by the other beam

Another method used is the recording of the ultrasoundcardiogram via a direct writing electrocardiograph (9) The time used by the sound to travel to the reflecting structure and back (transit time) is converted to a voltage signal which can be recorded by the electrocardiograph This conversion can be brought about by employing a condenser discharge for the measurements of the transit time Since the ultrasonic impulses are being sent and received at the rate of 1 000 per sec the resultant recording is essentially continuous This technique permits recording of the movements of only a single echo but it is possible to correlate the ultrasoundcardiogram with simultaneously recorded electrocardiogram and phonocardiogram (Fig 6)

The patients were examined in a supine position during normal respiration Most examinations were performed without premedication but a few anxious infants received a mild sedative Severely ill infants were examined within an incubator

RESULTS

Normal infants and children In all the cases examined we obtained echoes from the posterior wall of the heart and from the anterior mitral leaflet (Fig 2) The pattern of movement of the echo from the anterior mitral leaflet was similar to that observed in adults These echoes were obtained with the ultrasonic beam in antero posterior direction and with the transducer in the third or fourth left intercostal space and about half way between the left sternal border and the midclavicular line During

the early part of diastole there is a rapid inflow of blood into the left ventricle The echo from the anterior mitral leaflet makes a rapid posterior movement during this period The speed of movement of the echo from the anterior mitral leaflet during the early part of diastole was only measurable when the heart rate was slower than about 100 per min At a higher heart rate the duration of the part of diastole before the atrial systole is too short to allow measurement of the speed of movement The values for this speed of movement were between 90 and 140 mm/sec Sometimes we observed an echo with a small amplitude of movement anterior to the echo from the anterior mitral leaflet This echo probably arose from the interventricular septum

Echoes from the anterior tricuspid leaflet were obtained more easily in infants and children than in adults without heart disease These echoes could however only be obtained with the transducer directed in the medial cranial direction and placed in the fourth left intercostal space at the sternal border The pattern of movement resembled that obtained from the anterior mitral leaflet but in most cases the echo was only fragmentary

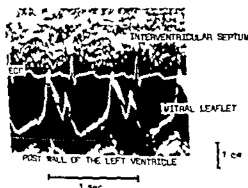


Fig 2 A B 10 years of age Normal mitral valve (no heart disease) UCG of the anterior mitral leaflet and the posterior heart wall with simultaneously recorded electrocardiogram As in all subsequent figures the anterior structures are in the upper part and the posterior structures in the lower part of the recording The posterior movement of the echo from the anterior mitral leaflet during the early part of diastole is rapid (135 mm/sec)

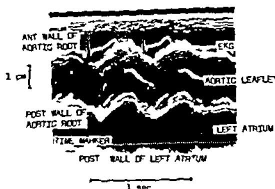


Fig 3 T B 8 years of age Normal aortic root (no heart disease) UCG of the root the left atrium and the posterior wall of the left atrium The recording is the same as Fig 1 B

With the transducer in the second or third left intercostal space and directed medially or occasionally slightly cranially we observed two parallel echoes assumed to arise from the anterior and posterior border of the outflow tract of the left ventricle more caudally or the aortic root more cranially (Fig 3) Occasionally we observed a faster moving echo between the two parallel echoes in the more cranial direction This echo moved towards one of the parallel echoes in the beginning of systole and away from it at the end of systole It has earlier been demonstrated that this echo originates from an aortic leaflet (4) Posterior to the two parallel echoes we observed an echofree space The posterior border of that space consisted of an echo with a small amplitude of movement and with a movement in presystole identifying it as an echo from an atrial wall This echofree space has been identified as representing the left atrium (11 13)

Congenital heart disease with dilatation of the right ventricle In children with various forms of congenital heart disease involving dilatation of the right ventricle we have always noted echoes from the anterior tricuspid leaflet and the interventricular septum These echoes were obtained with the transducer in the fourth or fifth left intercostal space at the sternal border and the ultrasonic beam directed antero-posteriorly (Fig 4) The pattern of

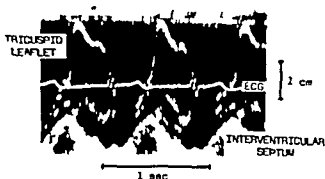


Fig 4 S W 13 years of age Atrial septal defect of secundum type UCG of the anterior tricuspid leaflet and the interventricular septum The recording was made with the transducer in the 4th left intercostal space at the sternal border and *in antero posterior direction*

movement of the echo from the anterior tricuspid leaflet corresponded to that obtained from the anterior tricuspid leaflet in adults This echo was in most cases observed continuously during both systole and diastole At a heart rate slower than 100 per min the speed of movement during the early part of diastole was measurable Excluding the cases of Ebsteins anomaly of the tricuspid valve the values for this speed of movement were between 55 and 160 mm/sec In some cases we observed echoes from two of the tricuspid leaflets The distance between the echo from the anterior chest wall and the echo from the interventricular septum was greater than normal for age in cases with dilatation of the right ventricle In the absence of dilatation of the right ventricle echoes from the anterior tricuspid leaflets were not obtained with antero posterior direction of the ultrasonic beam Echoes from the anterior tricuspid leaflet and the interventricular septum with an antero-posterior direction of the ultrasonic beam were noted in cases of atrial septal defects both of the ostium primum and secundum type the combination of atrial and ventricular septal defects very tight pulmonary stenosis Ebsteins anomaly of the tricuspid valve, primary pulmonary hypertension, some cases of ventricular septal defects combined with pulmonary stenosis a few cases of isolated ventricular septal defects and various forms of cyanotic congenital heart malformations

Congenital heart disease with dilatation of the left atrium In cases with severe dilatation of the left atrium the distance between the echo from the anterior mitral leaflet and the echo from the posterior wall of the heart was greater than normal A greater distance than normal between the echo from the posterior wall of the aortic root and the echo from the posterior left atrial wall was also found in these cases

Congenital malformations of the tricuspid valve Tricuspid atresia was the only condition

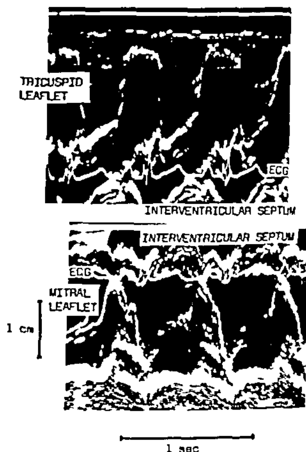


Fig 5 C S 6 years of age Ebsteins anomaly of the tricuspid valve *UCG of anterior tricuspid leaflet* and the interventricular septum (*upper part*) and the interventricular septum and the anterior mitral leaflet (*lower part*) Both recordings were obtained with the transducer in the 4th left intercostal space the lower part a little more to the left and both with the ultrasonic beam in antero posterior direction Note *the abnormally anterior position of the echo from* the anterior tricuspid leaflet during the entire diastole



Fig 6 M P 3 years of age Congenital mitral stenosis and coarctation of the aorta UCG of the anterior mitral leaflet with simultaneously recorded el ctrocardiogram and phonocardiogram before and after valvotomy The recordings were made with a direct writing electrocardiograph Note the abnormally slow speed of movement of the echo *from* the *anterior* mitral leaflet during the early part of diastole before valvotomy (25 mm/sec) *and* the *increase in* speed *of movement after operation 40–45 mm sec)*

in which we were unable to obtain any echo from the tricuspid leaflets

In 4 cases of Ebsteins anomaly of the tricuspid valve echoes from the anterior tricuspid leaflet and the interventricular septum were obtained with the ultrasonic beam in antero-posterior direction as in other forms of dilatation of the right ventricle The echo from the anterior tricuspid leaflet was however observed with the transducer placed more laterally on the precordium as far laterally as the left medioclavicular line The pattern of movement of the echo from the anterior tricuspid leaflet was during diastole characterized by a very slow posterior movement (<25 mm/sec) This resulted in an abnormally anterior position of the echo from the anterior tricuspid leaflet during the entire diastole (Fig 5)

Congenital malformations of the mitral valve In 2 cases of congenital mitral stenosis both combined with coarctation of the aorta the pattern of movement of the echo from the anterior mitral leaflet had a distinctly abnormal configuration (Fig 6) The total amplitude of movement of the echo was normal but the speed of movement in the early part of diastole was abnormally slow 20 and 25 mm/sec in the 2 cases After valvotomy the speed of movement in diastole increased to 35–45 mm/sec

In 2 cases of atresia of the mitral valve we observed echoes from the anterior tricuspid leaflet and the interventricular septum with the transducer in antero-posterior direction as in other cases with dilatation of the right ventricle Posterior to these echoes and 7–10 mm anterior of the echo from the posterior heart wall we observed an echo with a very small amplitude of movement (3–4 mm) its pattern of movement differed to that obtained from the mitral leaflet in all other cases (Fig 7) We have assumed that this echo represents the atretic mitral region In 4 cases with a hypoplastic left ventricle but with a mitral valve we also noticed echoes with a small amplitude of movement in front of the echo from the pos

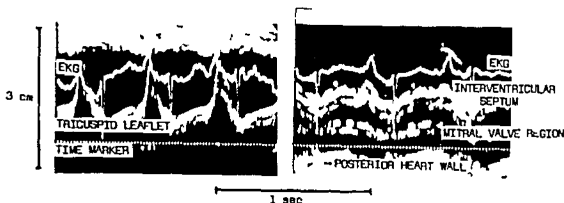


Fig. 7. A. L. A. 2 weeks old. Congenital mitral atresia. UCG of the anterior tricuspid leaflet (*left part*) and the interventricular septum, the mitral valve region and the posterior heart wall (*right part*). Both recordings were made in the 4th left intercostal space but left part closer to the left sternal border. Note the abnormal echo obtained from the mitral valve region instead of the normal echo from the anterior mitral leaflet.

terior wall of the heart. In these cases, however, the echoes from this region had a pattern of movement resembling that from a mitral leaflet. In one of these cases we even obtained an echo from the posterior mitral leaflet, thereby confirming the existence of a mitral ostium.

Four cases with atrial septal defect of the ostium primum type with cleft anterior mitral leaflet were examined. In three of them we observed abnormal echoes from the mitral region. Instead of the normal echo from the anterior mitral leaflet there were two parallel echoes, both with a pattern of movement typical for the echo from the anterior mitral leaflet. These echoes represented structures separated by a distance of 7 to 9 mm. It was possible on angiocardiography in the lateral projection to demonstrate the two parts of the cleft anterior mitral leaflet. The distance between the two parts measured on the angiocardiography was in close agreement with the distance between the two parallel echoes. One patient has been operated upon and at inspection of the valve a cleft of the anterior mitral leaflet was found and sutured. At postoperative investigations with UCG in this patient only a single echo from the mitral region was given.

In 2 cases of severe congenital mitral regurgitation we found an abnormally large amplitude of movement of the echo from the anterior mitral leaflet compared with that in normal children of the same age. The speed of movement during diastole was within the normal limits. In two other cases with mitral regurgitation where the regurgitation was judged as slight we noticed normal echoes from the mitral region. In another case of mitral regurgitation we found two parallel echoes from the mitral region in the same way as described in the 3 cases with a cleft anterior mitral leaflet and an atrial septal defect of ostium primum type. The electrocardiogram of this patient was typical for a case of endocardial cushion defect, but a catheterization failed to prove any atrial septal defect. A left ventricular angiogram demonstrated a deformed mitral valve and a mitral regurgitation but no cleft in the anterior mitral leaflet could be shown. From our observations on UCG we suspect, however, that this patient has a cleft anterior mitral leaflet of the endocardial cushion defect type.

We have investigated 3 cases of congenital corrected transposition of the great arteries with ventricular inversion. In all three we observed two parallel echoes in the position normally held by the echo from the anterior mitral leaflet. Both echoes had a pattern of movement typical for an echo from a leaflet of an atrio-ventricular valve. In this malformation there is a tricuspid valve between the left atrium and the arterial ventricle and a bicuspid valve between the right atrium and the venous

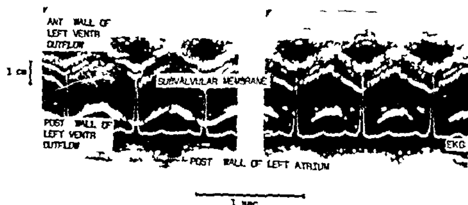


Fig 8 I K 6 years of age *Membranous subaortic stenosis* UCG from the outflow tract of the left ventricle Note the abnormal echo from the subvalvular membrane seen between the echoes from the anterior and posterior walls of the outflow tract of the left ventricle during both systole and diastole

ventricle We therefore assume that we registered echoes from two of the three leaflets of the tricuspid valve between the left atrium and the arterial ventricle

Congenital malformations with obstruction of left ventricular outflow Four cases of membraneous subaortic stenosis have been studied In all of them we obtained an abnormal echo between the two parallel echoes from the outflow tract of the left ventricle (Fig 8) This echo was observed both during systole and diastole and was easily distinguished from the echo of an aortic leaflet With a slight angulation of the transducer we could obtain both the abnormal echo (more caudally) and an echo from an aortic leaflet (more cranially) In two of these cases the echo from the anterior mitral leaflet was normal but in the other 2 cases the speed of movement during the early part of diastole was slightly reduced (60–70 mm/sec) The pressure gradient between the left ventricle and the aorta was lowest in the cases with the normal echo from the ant rior mitral leaflet

Several other cases with congenital obstruction to the outflow from the left ventricle (aortic stenosis isolated or combined with coarctation of the aorta) have been examined In 3 cases where the degree of obstruction was severe we noted an abnormal pattern of movement of the echo from the anterior mitral leaflet. The speed of movement during the early part of diastole was slower than normal (42 47 and 55 mm/sec) The echoes from the outflow tract of the left ventricle and the aortic root were normal

Primary myocardial disease Three patients with hypertrophic obstructive cardiomyopathy have been investigated They all showed an abnormal echo from the anterior mitral leaflet (Fig 9) The speed of movement during the early part of diastole was slower than normal

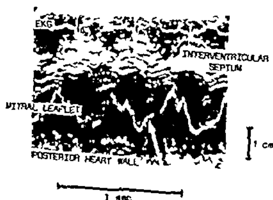


Fig 9 T M 2 years of age Hypertrophic obstructive cardiomyopathy UCG of the interventricular septum the anterior mitral leaflet and the posterior heart wall The speed of movement of the echo from the anterior mitral leaflet during the early part of diastole is somewhat slow (75 mm/sec) Note the abnormal anterior movement of the echo from the anterior mitral leaflet (*arrow*) during ventricular systole and the broad echo from the interventricular septum

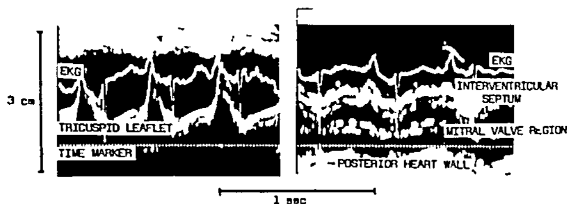


Fig 7 A L A 2 weeks old Congenital mitral atresia UCG of the anterior tricuspid leaflet (*left part*) and the interventricular septum the mitral valve region and the posterior heart wall (*right part*) Both recordings were made in the 4th left intercostal space but left part closer to the left sternal border Note the abnormal echo obtained from the mitral valve region instead of the normal echo from the anterior mitral leaflet

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increased speed of movement of the echo from the tricuspid leaflet This increased speed of movement was found to be reduced after operation In our material the speed of movement of the echo from the anterior mitral leaflet in cases of ventricular septal defect and patent ductus arteriosus as well as that from the anterior tricuspid leaflet in cases of atrial septal defects was found to be within normal limits Furthermore we have not been able to demonstrate a significant reduction after operation Our material of these heart lesions studied by UCG is however rather small and larger material has to be investigated for an accurate evaluation of UCG in estimating the degree of left to right shunting

Echoes from the tricuspid leaflet—with the tran ducer in antero-posterior direction—ve only been found in cases with a dilatation the right ventricle It is not clear whether is is due to the dilatation per se or to a multareous rotation of the heart For answer g this a comparative study of UCG and right entricular angiocardiography has to be per ormed The UCG technique seems however romising as a screening test for detection of ilatation of the right ventricle

In cases of Ebsteins anomaly of the tricuspid alve UCG has been found to be of particul rly diagnostic value The echo from the an erior tricuspid leaflet is in this anomaly obained far more laterally on the precordium than in other heart lesions with dilatation of the right ventricle and furthermore it has a different pattern of movement (17) resembling that described in cases of acquired tricuspid stenosis (1 15) There is however a difference in the pattern of movement of the echo from the anterior tricuspid leaflet in cases of tricuspid stenosis and in Ebsteins anomaly In tricuspid stenosis the movement of the echo from the anterior tricuspid leaflet in diastole is influenced by atrial systole In Ebsteins anomaly of the tricuspid valve the atrial systole causes no movement of the echo from the anterior tricuspid leaflet

In all cases except tricuspid atresia at least fragments of an echo could be obtained from the anterior tricuspid leaflet In doubtful cases a typical echo from a tricuspid leaflet therefore excludes the diagnosis of tricuspid atresia

Many cardiological centres have by now acquired considerable experience in the use of UCG for the diagnosis of mitral stenosis and evaluation of its severity A fairly high correlation has thus been found between the degree of stenosis of the mitral valve and the speed of movement of the echo from the anterior mitral leaflet during the early part of diastole (5 7 12) In 2 children with congenital mitral stenosis both combined with coarctation of the aorta the UCG findings are in agreement with those obtained in adults with acquired mitral stenosis Moreover the increase in speed of movement of the echo from the anterior mitral leaflet after valvotomy corresponds to the findings in adults (4 5 12)

Mitral atresia is the only condition where we have not succeeded in obtaining an echo with the pattern of movement typical for that from the anterior mitral leaflet Instead an abnormal echo was obtained with a pattern of movement similar to that described in normal subjects and patients with acquired mitral disease by Zaky et al (27) they assumed that this echo originates from the mitral ring region This observation together with the UCG findings in other cases of the so-called hypoplastic left heart syndrome indicate a new adjunct in the methods of diagnosing this group of congenital heart disease and particularly mitral atresia

Sommerville (24) has pointed out that mitral regurgitation is one of the important factors determining the natural history of endocardial cushion defects Demonstration of an abnormal mobility of the mitral leaflets and of a cleft anterior mitral leaflet are important for the diagnostic evaluation of cases with atrial septal defect of the ostium primum type UCG can therefore contribute to a better understanding of the function of the mitral valve in these cases The prerequisite for obtaining double echoes from a cleft mitral leaflet is of course that the two parts of the leaflet are

(40, 70 and 75 mm/sec) In one of these patients showing a speed of movement of 70 mm/sec a septomyotomy was performed and after the operation the speed of movement increased to 85 mm/sec The most important abnormal finding in these patients was however, an anterior movement of the echo from the anterior mitral leaflet during ventricular systole, thus narrowing the outflow tract of the left ventricle This anterior movement was most pronounced in the case with the greatest pressure gradient between the left ventricle and the aorta This abnormal movement could best be seen by registration of the echo from the anterior mitral leaflet in the usual way It could however also be seen at least in the most severe cases when the transducer was directed towards the lower part of the outflow tract of the left ventricle The echo from the interventricular septum was unusually broad in these cases with hypertrophic obstructive cardiomyopathy

In 3 cases with severe acute myocarditis normal echoes from the anterior mitral leaflets were registered except in one of the patients In the initial stage with severe congestive heart failure this patient showed a slightly reduced speed of movement of the echo from the anterior mitral leaflet during the early part of diastole (75 mm/sec) but this later became normal

Pericardial effusion In 4 cases with pericardial effusions an echofree zone between the echo from the anterior structures presumably also including the parietal pericardium, and that from the anterior heart wall was found In two of them an echofree zone posterior to the echo from the posterior heart wall was also found After pericardiocentesis the echofree zones disappeared

Atrial thrombosis In 1 patient abnormal echoes were found posterior to the echo from the anterior tricuspid leaflet and anterior to the echo from the posterior heart wall These registrations were made with the transducer in the fourth left intercostal space parasternally and placed in medio cranial direction This space posterior to the echo from the anterior tricuspid leaflet was later confirmed to be the right atrium by obtaining dense echoes in this area at injection of indiocyanine green using the method described by Gramiak et al (11) The abnormal echoes obtained without contrast injection were stratified and moved in and out of the ultrasonic beam with each heart beat Angiocardiography confirmed the presence of a mass presumably a thrombosis, in the posterior part of the right atrium This patient had earlier been operated upon twice with insertion of Spitz Holter catheters for hydrocephalus These catheters had been removed because of malfunction

DISCUSSION

Principally the same structures as in healthy adults can by UCG be studied in infants and children without heart disease Echoes from the anterior tricuspid leaflet are however more easily obtained in younger individuals than in adults As in adults a complete UCG study of the mitral valve requires a sufficiently long interval between the end of ventricular systole and the beginning of atrial systole The speed of movement of the echo from the anterior mitral leaflet during the early part of diastole was measured to 90–140 mm/sec i e the same values as in adults (3)

In cases of atrial septal defect ventricular septal defect and patent ductus arteriosus Jacobi et al (14) found for each defect a typical pattern of movement of the echo from the anterior mitral leaflet Like Schmitt et al (20) we have been unable to confirm this observation

In cases of ventricular septal defect and patent ductus arteriosus with left to right shunting Ultan et al (26) found during the early part of diastole a more rapid movement of the echo from the anterior mitral leaflet than in healthy subjects This has been attributed to an increased flow through the mitral ostium In cases of atrial septal defects with left to right shunting the same authors found a similarly

rior movement of the echo from the anterior itral leaflet during ventricular systole and on e other the systolic murmur and the con guration of the arterial pressure curve in ese cases as well as the effect of pharmaco gical tests on all these parameters UCG erefore seems well established as a diagnostic ool in cases of hypertrophic obstructive car iomyopathy and in addition it provide val able information on the variability of the out low obstruction in this disorder

The usefulness of UCG for demonstration f pericardial effusion has previously been re ported by many authors (2 9 16 19) Our observations confirm this and thus show that the UCG technique is for this purpose also applicable on infants and children

Several authors have reported that tumour or thrombosis can give abnormal echoes (2 8) most of these have been demonstrated from the left atrium Our UCG studies in a patient with thrombosis in the right atrium show that information may also be obtained from the right atrium This examination is especially valuable in patients operated for hydrocepha lus with the Spitz Holter method

Pronounced deformity of the thoracic wall makes the use of UCG difficult due to the need for air free contact between the trans ducer and the chest wall The use of a smaller transducer can solve some of these problems Anomalies of the position of the heart makes the interpretation of the UCG difficult the use of the contrast method as described by Gra miak et al (11) facilitates however the identi fication of echoes and echofree spaces within the heart

The danger of tissue damage from the use of ultrasound for diagnostic purpose has been discussed by several authors (3 23) No tissue damage has however so far been discovered following investigations with reflected ultra sound in the form used here This is also un likely to happen due to the small amount of energy produced by this technique with its very short pulses of ultrasound

One of the advantages of the UCG tech nique is that it offers a simple and fairly rapid examination which can easily be repeated It can even be used in small seriously ill infants also these lying in an incubator The method is of great value as a complement to the or dinary methods for investigation of infants and children with heart disease

SUMMARY

Ultrasoundcardiography (UCG) studies have been performed in about three hundred chil dren The age of the children varied between 1 day and 16 years Most of the studies have been performed without premedication and all without discomfort

The UCG technique has been found valu able for demonstration of dilatation of the right ventricle both in cyanotic and acyanotic congenital heart disease In studies of lesion affecting the tricuspid valve pathological UCG findings have been obtained in patients with tricuspid atresia and Ebsteins anomaly

The method has also been found useful in evaluation of the state of the mitral valve Pathological findings have been obtained in congenital mitral stenosis mitral atresia and other forms of hypoplastic left heart syndrome in endocardial cushion defects and in mitral regurgitation

The UCG technique also provides informa tion about the outflow tract of the left ven tricle Pathological findings have been obtained in cases with membraneous subvalvular aortic stenosis and hypertrophic obstructive cardiomyopathy

Pericardial effusion and thrombosis in the right atrium may be demonstrated by this tech nique No complications have been observed

ACKNOWLEDGEMENT

This work was supported by a grant (to N R L) from the Swedish National Association against Heart and Chest Diseases

located at different distances from the anterior chest wall On the other hand it must be stressed that the finding of double echoes from the mitral region is not synonymous with a cleft mitral leaflet, this has clearly been dem onstrated in our studies of patients with congenital corrected transposition of the great ar teries with ventricular inversion In cases with abnormally thick mitral leaflets a double echo from the anterior mitral leaflet can sometimes be found In these cases is, however, the dis tance between the two parts less than that obtained in cases with cleft anterior mitral leaflet

Our observations in patients with severe mi tral regurgitation of a large amplitude of movement of the echo from the anterior mitral leaflets are in agreement with the UCG findings reported in cases of acquired mitral regurgitation (5 21)

The echofree zone between the echo from the anterior mitral leaflet and that from the posterior heart wall has been assumed to represent the left atrium in most cases (4) This has by means of contrast studies now been confirmed by Gramiak et al (11) In cases of severe dilatation of the left ventricle and with a more caudal direction of the ultrasonic beam this zone will instead represent the left ven tricle These two possibilities can easily be dif ferentiated from each other by noting the different pattern of movement of the echo from the left atrial wall as compared with that from the left ventricular wall the echo from the pos terior left atrial wall moves in an anterior di rection during presystole while the echo from the posterior left ventricular wall moves in an terior direction during systole The echofree zone posterior to the posterior wall of the left ventricular outflow tract or the aortic root gives however, a more reliable presentation of the left atrium and permits also a rough estimate of the size of the left atrium A more accurate measurement may however be made by the technique suggested by Hirata et al (13)

Ultan et al (26) have described an echo presumably representing a membraneous sub aortic stenosis and they have also presented certain criteria for the identification of this echo In such cases we have observed a similar echo within the outflow tract of the left ven tricle This echo does not resemble that from an aortic leaflet, the latter is most often seen between the two parallel echoes from the aor tic root The UCG findings in patients with membraneous subaortic stenosis are also dif ferent from those obtained in cases of hyper trophic obstructive cardiomyopathy We would however, like to add one more criterion to those given by Ultan et al (26), the echo from a membraneous subaortic stenosis must be identified between the two parallel echoes from the outflow tract of the left ventricle or be anterior to the echo from the anterior mitral leaflet, and, furthermore, clearly separated from the echo from the interventricular sep tum If these criteria are not fulfilled the ech oes from the mitral ring region or the inter ventricular septum may be misinterpreted as coming from a subvalvular membrane

A reduced speed of movement of the echo from the anterior mitral leaflet during the early part of diastole has been demonstrated in cases of severe valvular aortic stenosis (5) This is presumably due to an impaired inflow to the left ventricle secondary to a reduced compli ance The same mechanism is presumably also responsible for similar findings obtained in pa tients with hypertrophic obstructive cardio myopathy Stewart et al (25) have demon strated in patients with hypertrophic obstruc tive cardiomyopathy and valvular aortic steno sis a reduced compliance of the left ventricle

The anterior movement of the echo from the anterior mitral leaflet observed during ventric ular systole in cases of hypertrophic obstruc tive cardiomyopathy seems to be typical for this disease This abnormal movement of the anterior mitral leaflet explains probably the mitral regurgitation often seen in these cases Similar observations have recently been pub lished by Shah et al (22) and Pridie et al (18) Shah et al (22) have clearly demonstrated the relation in time between on one side the an

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Table 1 *Laboratory data of case II*

	Age (hours)								
	72	90	98	118	138	144	146	162	174
Serum K (mEq/l)	8 2	9 1	6 9	7 4	4 8	5 0		6 2	
Serum Na (mEq/l)	131	139	139	134	130	132		142	
Serum Cl (mEq/l)	97	98	93	92	85	77		102	
pH	7 00	7 00	7 09	7 04	7 04	7 03	7 12	7 38	7 19
St bicarbonate (mEq/l)	8	8	10	12	14	14	16	17	14
Pco (mmHg)	32	30	30	59	85	70	58	22	36
O_2 saturation ( )			96	98				100	
Urea (mg/100 ml)		99		165	199			147	
Creatinine (mg/100 ml)					4 4				
Blood glucose (mg/100 ml)	180								
Serum Ca (mEq/l)		4 2			4 3				2 6
Serum Mg (mEq/l)					1 8				1 5
Prothrombin test ( )				15					
Albumin (g/100 ml)					4 0				
Bilirubin (mg/100 ml)					4 4				
SGOT (U/l) (normal 4–20)		71							
SGPT (U/l) (normal 2–17)									
LDH (U/l) (normal < 200)		1 800			2 300				
HBDH (U/l) (normal < 125)		1 380			1 615				
CPK (U/l) (normal < 35)		660			296				
Hgb (g/100 ml)	15 5								
WBC (No per μl)	11 400								
Lactic acid (mg/100 ml blood)			97	88			97		109
Pyruvic acid (mg/100 ml blood)				3 3					

Laboratory investigations (68 hours of age) Hgb 18 8 g per 100 ml RBC 5 26 millions per μl hematocrit 60 WBC 30 200 per μl nucleated red cells 3 per 100 WBC thrombocytes 169 000 per μl prothrombin test 10 blood sugar 30 mg per 100 ml Serum electrolytes (mEq/l) potassium 8 2 sodium 134 chlorides 113 calcium 4 4 Acid base values pH 7 04 standard bicarbonate 9 mEq/l PCO_2 22 mmHg Spinal puncture yielded a yellow cloudy fluid with a cell count of 1 per μl spinal fluid glucose 13 mg per 100 ml spinal fluid protein 288 mg per 100 ml

The clinical picture was consequently that of a severe metabolic acidosis with elevated serum potassium levels A septic etiology could not be excluded and the child was given penicillin sulphonamides and chloramphenicol There was no response to the treatment The acidosis was persistently severe and the serum potassium level remained high Increasing spasticity and signs of cerebral degeneration characterized the development Determinations at 94 hours of age showed serum potassium at 8 1 mEq/l sodium 138 mEq/l chloride 92 mEq/l pH 7 01 standard bicarbonate 9 mEq/l and PCO 35 mmHg A severe sceredema developed and the child expired at the age of 100 hours No examinations were made in order to find organic acids in the serum which could explain the acidosis

Case II I L S (born April 2 1968)
The pregnancy was uneventful with delivery 20 days after term of a girl with birth weight 3 480 g length 55 cm The child cried immediately and appeared healthy Placenta was normal During the second day of life the child had some vomiting and appeared slightly dehydrated On April 5th the child was admitted to our department

On admission the child was nearly 72 hours of age She had a dysmatur appearance with loose wrinkled skin Truncus was long and thin There were definite signs of cerebral damage including hypertonicity and high pitched cry The respiration rate was somewhat increased (48/min) and on auscultation some coarse rales could be heard over both lungs

The results of laboratory investigations are presented in Table 1 Spinal puncture revealed a yellow fluid with a cell count of 3 per μl spinal fluid glucose 40 mg and spinal fluid protein 218 mg per 100 ml Bacteriological examination of the spinal fluid was negative Amino acid chromatography of urine and serum did not reveal any significant deviations from the normal picture The urine was acid and contained protein as well as ketone bodies

According to Table 1 the child suffered from a severe metabolic acidosis with an elevated serum po

Fig 1 Case I O B S Section through cerebellum showing bilateral cystic spaces protruding into the hilum of the dentate nuclei × 16 Weil staining

Fig 2 Case I O B S Cerebral white matter transition to gelatinous emicystic area Loose spongy structure with few cells × 160 Luxol fast blue

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Table I *Laboratory data of case II*

	Age (hours)								
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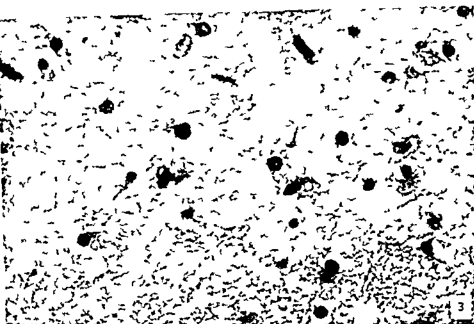
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4

Fig 3 Case I O B S Swollen cytoplasm in glial cells rounded or stellate ×640 Luxol fast blue

Fig 4 Case I O B S Pale staining stellate glial cells in spongy semicystic area ×640 Luxol fast blue

tassium Calculation of the available electrolyte values *suggested an accumulation of some organic acid* in the blood Lactic acid and pyruvic acid showed 88–108 mg lactate per 100 ml of blood (9 8–12 mMol per l) and 3 3 mg pyruvate per 100 ml (0 4 mMol per l) A ratio of lactic acid to pyruvic acid of 24 to 1 was *found Gas chromatography of urine* showed lactate values from 68 to 434 mg per 100 ml The diagnosis of a severe lactic acidosis was *thus confirmed*

Treatment was started with antibiotics (penicillin streptomycin and colimycin) and intravenous fluid *including isotonic bicarbonate* The acidosis persisted and even rapid injections of hypertonic bicarbonate did not have any significant effect on blood pH

The elevated level of serum potassium was confirmed by repeated determinations (Table 1) Reduction of the high levels was achieved by the combined use of resonium by mouth and by adding glucose with insulin to the intravenous program Glucose was the only source of calories given to the patient the first days after admission However as the need for parenteral nutrition persisted amino acids were given together with lipids (Intralipid®) Neither of these regimen seemed to influence the production of organic acids in the body

The general condition of the infant deteriorated rapidly and scleredema became obvious Cerebral damage became more pronounced and from the fourth day of life the child presented with no motoric activity except for respiration An exchange transfusion was performed on April 8th as a last attempt to alleviate the acidosis and to clear the body of the accumulating metabolites During this procedure however the child got apnoea and had to be put *on a respirator (age 144 hours)* The acid base values showed an improvement after this however with a pH the next day of 7 38 standard bicarbonate 17 *mEq/l and PCO_2 22 mmHg However th* clinical condition remained unchanged At the age of 177 hours the child died from acute occlusion of th airways

Autopsies

The pathological findings in the two siblings were *nearly identical and restricted to the* lungs the liver, the thymus and the brain They are here described together

The lungs were atelectatic with small hemorrhages

The liver had a yellowish brown colour size and consistency were not remarkable Microscopically a few hematopoietic foci occurred The liver cells were vacuolated and frozen sections from the second case *showed them* filled with Sudanophilic material (tissue for frozen sections *from visceral organs* of case I was not preserved) Paraffin embedded sections showed PAS positive loading in the liver cells, mainly around the central veins

The kidneys were normal, but in frozen sections from case II Sudanophilic material was also found in the epithelial cells of convoluted tubuli

The thymus was in both cases small (weight 5 g and 2 5 g) Cortex was nearly depleted of lymphocytes, and contained large cells filled with Sudanophilic droplets and also PAS positive granules These changes were mostly marked in the second case

The brain (weight 550 and 530 g) One hemisphere from case II was immediately after removal preserved deep frozen, otherwise the brains were fixed in 10% neutral formalin for about 14 days before cutting The hemispheres were symmetrical and the gyri well developed The consistency was soft Coronal sections showed widening of the lateral ventricles Subependymal cystic spaces were seen lateral to the side ventricles and separated from them only by a thin membrane Similar cystic spaces were *seen also in the cerebellum* localized to the hilum of the dentate nuclei (Fig 1) The cut surfaces showed white to greyish gelatinous sunken areas mainly localized to the central parts of the white matter, mostly pronounced in frontal and temporal regions *Microscopically* the subependymal cysts were lined by a few layers of organized glial cells The ependymal lining of the ventricles was well preserved The cortex and central gray substance showed a development as expected in *newborn infants* The white substance and e pecially the central parts had a spongy structure (Fig 2) It was poor in cells and those present were stellate astroglial cells with varying amounts of visible cytoplasm which in frozen sections contained small Sudanophilic granules (Figs 3 and 4) These were more numerous in the be t preserved periventricular areas and absent in the looser gelatinous parts Rounded eosinophilic cells were also conspicuous PAS staining of the brain was negative

Inflammatory cells were not present in any

Clinical course
Died 3½ years of age
Improved
Died 2 years of age
?
Improved
Improved
Died 2 years of age
Died 2 years of age
Died 16 months of age
Observed till 7 months
Died 7 months of age
Observed till 4 years of age
Died 30 months of age

biochemical defects No enzymatic deficit has so far been demonstrated in any patient.

We feel that the 2 patients described in the present report constitute a further subgroup of chronic lactic acidosis as they differ in several respect from those previously reported

Clinical course The disease manifested itself during the second and third day of life The early signs were slight dehydration irritability and signs of cerebral damage Convulsions were never observed The cerebral damage was reflected through an increasing hypertonicity and elimination of spontaneous movements of the extremities Death occurred at the age of 100 and 174 hours

One of the common signs among the children with lactic acidosis has been episodes of hyperpnoea associated with ri e in blood lactate Our patients might have had a slight hyperventilation early in life indicated by the low PCO detected in the first patient on admission However no real respiratory effort to get rid of the acid load could be detected clinically and the PCO values increased with time One explanation of this could be a damage to the respiratory center along with the general cerebral damage that obviously progressed as the metabolic acidosis persisted None of the previously reported cases of hyperlactatemia of infancy has showed such a malignant course

Biochemical findings Serum potassium levels were elevated in both patients In the children previously described where this has been mentioned normal values have been found A possible explanation of this elevation could be renal failure although renal function was not markedly reduced early in the disease The acidosis could also explain some of the elevation as an accumulation of H ions can cause a shift in potassium from the intracellular to the extracellular phase

Aminoaciduria could not be detected in our patients but has been described by others (6 9 20) Those who have examined the spinal fluid have found normal liquor Both our patients had a marked increase in spinal protein Even though the protein content of the spinal fluid normally is somewhat elevated in the neonatal period the elevation demonstrated was clearly pathological The increase is compatible with a process of severe damage to nervous tissue

The level of lactic dehydrogenase (LDH) was greatly elevated in the serum of the second sibling This increase has been found in children with congenital lactic acidosis (9) as well as in the idiopathic acidosis of adults (17) The isoenzyme pattern suggests that the excess LDH is of hepatic origin (16) The levels of creatine phosphokinase were elevated as well in the second sibling This could point to muscle damage but can also be seen where there is destruction of nerve cells Details of biochemical investigations performed on the second patient can be found in the accompanying paper (16)

Autopsy findings The changes were close to identical in the two siblings Both livers were heavily stored by lipid and ome lipid was also disclosed in the tubular epithelium in the kid

Table 2 *Chronic lactic acidosis in children Data on previously reported cases*

Authors	Sex	Birth weight	Age at start of hyperpnoea	Age at diagnosis	Hypo tonicity	Convul siones	Mental retard ation	Serum potassium	CSF[a]
Hartmann et al (1962)	♀	2 600	Early infancy	4 months	+	+	+	N[b]	?
Nordio et al (1963)	♂	3 000	10 days	16 days	?	+	–	?	?
Israels et al (1964)	♀	?	8 months	11 months	+	+	+	?	?
	♂	2 100	4 months	9½ months	+	+	+	N	N
Erickson (1965)	♂	3 530	1 day	3 weeks	+	+	+	N	?
	♀	?	1 day	17 months	+	+	+	N	?
Worsley et al (1965)	♂	3 175	18 months	17 months	+	+	+	N	N
	♂	3 855	4 months	19 months	+	+	+	N	N
Haworth et al (1967)	♂	?	3 months	3 months	+	–	+	N	N
	♂	3 840	1 day	3 days	+	+	+	N	?
Schärer et al (1968)	♂	2 850	8 days	5 months	+	+	+	N	N
Bejar et al (1968)	♂	?	Never had	4 years	–	–	–	?	?
Peytel et al (1969)	♂	2 900	First months of life	8 months	+	+	+	N	N

[a] Cerebrospinal fluid
[b] Normal values

part examined Myelin stained sections showed no myelin in the hemispheres the internal capsules or in the cerebellum Around the basal ganglia and brain stem a few myelinated fibers were present The spinal cords revealed faintly staining myelin in the posterior columns and ascending sensory tracts The root fibers all stained well and could be followed to their nuclei in the cord and brain stem The axis cylindres were scarce and appeared fragmented in the unmyelinated hemispheres

DISCUSSION

Two siblings have been described who developed a severe metabolic acidosis during the first days of life In the second sibling the acidosis was demonstrated to be caused by an accumulation of lactic acid in the serum In the first sibling no lactic acid determinations were made However, the similarities in clinical picture the almost identical biochemical disturbances and autopsy findings combine very strongly to suggest an identical condition The fact that they were siblings suggest that genetic factors are of importance As to the etiology of the hyperlactatemia we were unable to disclose any of the known conditions which may give lactic acidosis (see p 124) We therefore consider our two patients to suffer from a primary type of lactic acidosis in which biochemical lesion most probably is present

Thirteen patients have so far been described in the literature with chronic hyperlactatemia in early infancy and childhood Some of the clinical and biochemical features of these patients have been summarized in Table 2 The common denominator in all has been the demonstration of a chronic metabolic acidosis caused by an accumulation of lactic acid in the serum In three of the reports (2 6 20) the patients described have been siblings suggesting a genetic basis for the disease The clinical course in the individual patients, however has varied considerably suggesting that the hyperlactatemia can be caused by different

mination of lactic acid was performed but the similarities in clinical biochemical and pathological findings combine very strongly to suggest an identical condition

The children died at the age of 100 and 177 hours The main pathological findings were confined to the brain the thymus and the liver Softening of the white matter was pronounced with delayed myelination Organized bilateral paraventricular cystic spaces were found Fatty changes were pronounced in the liver

In some respects these children differ from those previously reported with congenital lactic acidosis

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neys where frozen sections were examined Whether this storage can be connected with the metabolic disturbance possibly responsible for the cerebral damage cannot be decided

Both patients had small thymus glands with few lymphocytes and many macrophages containing lipid and mucoprotein material As no immunological study was possible the question of the role of the thymus gland in this context must also be left open

The immediate impression gained on gross examination of the brain was the similarity to the picture described as sway back in lambs Winkelman & Moore (19) found the same likeness in the brain from a 12 week old child in whom a metabolic disturbance had not been considered Their diagnosis was a degenerative encephalopathy

Our 2 cases had only lived for a few days, and myelin formation is at that time only starting in the hemispheres Compared to the normal pattern, however our cases were definitely behind in development In both cases the subependymal cysts showed an organization in their walls that pointed to a process started during the intrauterine period No signs of hemorrhage could be found There were no reasons for assuming anoxia to have caused the lesions since both pregnancy and delivery were uncomplicated The cortex was well organized, which gives us reason to presume that whatever had interfered had acted during the last 2 months Considering the importance of oligodendroglia in the formation of myelin it seems justified to locate the deficient process to these cells

Worsley et al (20) described two siblings with lactic acidosis one of whom died at the age of 24 months They found changes in both the grey and white matter the first mainly located to diencephalon, reticulate substance and the brain nerve nuclei They labelled their cases as necrotising encephalopathy Even considering the different development in their case and ours, it is difficult to imagine a similar distribution of lesions if our patients should have survived

Scharer et al (15) described 1 case of lactic acidosis living for 7 months Their findings are more compatible with the present cases with lack of myelin in frontal parts of the brain the internal capsules and corpus callosum as well as a marked subependymal gliosis in pons cerebellum and around the 3rd ventricle They also found changes in cerebellum although myelin was present Peytel et al (13) reported 1 case of lactic acidosis living for a few months They found bilateral ventricular dilatation and frontal atrophy A pallidal necrosis was ascribed to intoxication by CO occurring as a consequence of serious acidosis Their findings may be in conformity with ours as the process not necessarily must work with the same speed in all cases

Poser (14) discussed two types of dysmyelinating disease the myelinoclastic and the dysmyelinogenic In the first myelin is destroyed by exogenous or endogenous factors in the second group myelin is not produced due to various enzymatic deficiencies He also pointed to the differences in myelin of the peripheral and central nervous system and the possibility of different types of myelin even within the brain that might be responsible for the variable location of lesions in different reported cases We do not think it is possible to determine whether enzymatic defects or toxic substances are the cause of the deficiency in myelin observed in our patients However, attention should be drawn to 1 case of fructosemia (10) where the pathological findings were quite similar to tho e presented in our 2 patients Although the enzymatic defects are not identical an interference with glial metabolism may have resulted in similar morphological picture

SUMMARY

Two siblings have been described who developed a fatal metabolic acidosis during the first days of life The acidosis was found to be caused by an accumulation of lactic acid in the second sibling In the first sibling no deter

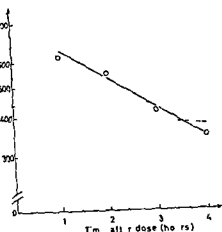


Fig 1 Serum radioactivity after the injection of lactate ? C The stippled curve represents the calculated decay curve caused by metabolism (See the text for further details)

Estimation of glycogen The glycogen content was determined essentially by the method of Hultman (16) Glycogen standards were carried through the whole procedure in each separate analysis

The radioactivity incorporated into glycogen during the *in vivo* as well as in the *in vitro* studies was determined upon its isolation (16)

Estimation of LDH isoenzymes Electrophoresis was performed in agarose gel (1%) in barbital buffer pH 8 6 and the fractions were visualized according to Barnett (3) The relative distribution of the isoenzymes was calculated from values obtained by scanning

Incubation experiments Liver and muscle (diaphragm) slices of 0 5 mm thickness were made with a Stadie Riggs microtome As incubation medium was used a Krebs-Ringer phosphate buffer pH 7 4 containing in mM NaCl 127 KCl 5 10 CaCl 2 73 MgSO 1 ?7 KH PO 11 2 To 3 ml of the buffer in a 50 ml Erlenmeyer flask were added 0 3 to 0 5 g of the tissue slices and 10 μmoles of 1 C lactate or 1 C pyruvate The flask was stoppered and incubated in a shaking water bath at 37 C for 1 hour After incubation 0 5 ml of 6 N HCl was added and the CO was trapped in 1 ml of Hyamine hydroxide in a liquid scintillation counting vial

Liver mitochondria were prepared by homogenization in 10 % sucrose with 0 5 mM EDTA and centrifugations according to Myers & Slater (?3) The oxygen uptake was measured with conventional Warburg techniques

RESULTS

In vivo studies

After the i v injection of lactate 2 ^{14}C (about 5 μCi) a nearly exponential disappearance of the serum radioactivity was found the rate constant of which was about 0 23/hour ($T/2$ = 3 hours)(Fig 1)

Only insignificant radioactivity was recovered in the urine in 4 ½ hours (Table 1) amounting to a total of about 0 45 % of the injected dose The excretion was considerably lower during the first hour after the injection than in the following 3 hours despite the fact that the serum radioactivity decreased exponentially during the study (Fig 1) This suggests that urinary lactate derived from tubular excretion rather than glomerular filtration

Table 2 shows the amounts of radioactive CO expired in periods of 5 min at 1 2 3 and 4 hours after the injection of lactate 2 ^{14}C From these values it can be estimated that

Table 1 *Urinary excretion of lactate and ^{14}C lactate*

Sample period (hours after dose)	Lactate excretion (mg/hour)	Radioactivity (cpm/hours)	(cpm/mg lactate)
0–1	1 68	370	220
1–3	3 40	13 200	3 880
3–4½	3 56	9 660	2 720

Table 2 *Expiratory $^{14}CO_2$ and CO_2*

Time of sampling (hours after dose)	$^{14}CO_2$ expired (counts/5 min)	CO expired (mmoles/5 min)
0	0	4 1
1	12 5?0	3 6
2	19 300	4 0
3	19 850	4 3
4	15 350	3 6

Table 3 *Radioactivity in various tissues post mortem*

Tissue	Radioactivity (cpm/g tissue w w)
Liver	2 780
Muscle	2 520
Heart	2 780
Brain	2 160
Kidney	3 000
Serum	2 200

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FATAL CONGENITAL LACTIC ACIDOSIS IN TWO SIBLINGS

II *Biochemical Studies in vivo and in vitro*

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A preceding paper (19) dealt with the results of clinical investigation and autopsy findings in two newborn siblings with congenital lactic acidosis Thirteen similar cases have been de scribed till now (4, 8 10 12, 17 24, 25 29, 33), but a common cause of the accumulation of lactic acid has not been revealed The present report deals with *in vivo* as well as *in vitro* studies carried out in an attempt to localize the metabolic defect in one of the children

METHODS

Chemicals Lactate 1 ^{14}C pyruvate 1 ^{14}C and lactate 2 ^{14}C were obtained from the Radiochemical Center Amersham England Caprinylcarnitine was a gift from Dr Jon Bremer All other reagents were com mercial products of high purity

Radioactivity assays The radioactivity was meas ured in a Tricarb liquid scintillation counter The counting efficiency for ^{14}C varied from 40 to 55 % but all the values presented are deduced to the same counting efficiency

In vivo studies

At the age of 7 days a single tracer dose of 10 μCi L lactate 2 ^{14}C was given intravenously Blood samples were taken at intervals Urine was quanti tatively collected and expiratory CO was sampled as described below In the course of the study there was no sign of circulatory failure and the respira tion was controlled artificially However 4 ½ hours after the injection the patient died due to acute occlusion of the airways

Estimation of radioactive and cold lactate pyru vate and glucose in serum Lactate and pyruvate in blood was estimated by enzymatic methods ^{14}C ac tivity in lactate and glucose was estimated in serum samples after precipitation of proteins with ethanol Supernatants were subjected to thin layer chromato graphy on silica gel (Kieselgel G Merck Darm stadt Germany) The elution mixture was *n* propa nol/ethyl acetate/water 55/35/10 by vol In this system glucose had an R_F of approximately 0 40 and the R_F value of lactate was 0 15

Estimation of total CO_2 and $^{14}CO_2$ Artificial re spiration by a respiratory pump was instituted at the age of 4 days due to respiratory failure During the *in vivo* study with lactate 2 ^{14}C expiratory air was collected directly from the outlet of the expiratory pump into rubber bags for periods of 5 min prior to the injection and after 1 2 3 and 4 hours The CO_2 in the collected air was absorbed in a 5 N NaOH solution CO_2 corresponding to 12 sec of res piration was quantitatively transferred by diffusion into 1 5 ml of Hyamine hydroxide 10-X (Packard) in a counting vial and the radioactivity was deter mined after addition of the liquid scintillator Th total CO_2 in the samples was determined as described by Stokke et al (30)

Urine analyses Urinary lactate was determined in a Perkin Elmer gas chromatograph model 800 at 114 C after extraction with diethyl ether and methy lation with diazomethane liberated from N nitrosom ethyl urea The stainless steel column (6 × 1/8) was filled with 8 % of butanediol succinate on Chro mosorb W (80-100 mesh) Carrier gas was N_2 with a flow of 25 ml/min Malonic acid dimethyl ester was used as an internal standard

In vitro studies

In the patient as well as in a control infant a partial autopsy was performed half an hour after their death The control patient suffering from multiple malfor mations died at the age of 8 days The tissues were chilled to 0 C or frozen at − 20 C immediately after the removal

Table 4 *The metabolism of lactate and pyruvate in liver and muscle slices*

Tissue slices (300-500 mg w w) were incubated in Ringer phosphate saline solution at pH 7 4 for 1 hour at 37 The final volume was 3 5 ml The values are mean of two parallels. The content of liver glycogen at the start of the experiment was 1 6 g/100 g in tissue from the patient and 5 6 in the control tissue

Substrate (μ moles)	Liver slices CO₂ formed (nmoles/g w w)		Incorporated into glycogen (nmoles/g w w)		Glycogen content at the end of incubation (g/100 g w w)		Muscle slices CO formed (nmoles/g w w)	
	Pat	Contr	Pat	Contr	Pat	Contr	Pat	Contr
Lactate 1 C (10)	2 116	760	7	33	0 5	1 5	3 430	657
Pyruvate 1 C (10)	4 940	1 460	12	63	0 6	—	5 740	1 880

the relative contribution of the oxidation to the total lactate metabolism From the values obta n~d for radioactive CO expiration when steady state condition was reached (Table 2) from the specific activity of lactate in the body and from the metabolic turnover rate for lac tate it can be calculated that the oxidation to CO_2 accounts for about 60° of total lactate removed

In vitro experiments

Tissue slices Table 4 shows that when liver slices obtained at partial autopsy were incu bated with pyruvate and lactate significant amounts of ^{14}CO were produced The ^{14}CO formation was about twice as great with pyru vate as with lactate With both substrates however the ^{14}CO production was significantly higher in the slices from the patient than from the control Since lactate was equally efficient as substrate in the patient as in the control a defect in LDH was apparently not present Only small amounts of the radioactivity wa recovered in the liver glycogen at the end of the incubation about 5 times more in the slices from the control than from the patient In the liver slices from both infants the glycogen con tent was considerably reduced during the in cubation showing that no net gluconeogenesis had occurred Thus as expected from the in cubation conditions chosen (11), glycolysis and oxidation dominated over gluconeogenesis in the experiments with liver slices Muscle slice from the ptatient and the control showed a pat tern of oxidation similar to liver slices when incubated with radioactive lactate and pyru vate (Table 4)

Table 5 *Oxidation of pyruvate by liver mito chondria*

Liver mitochondria were incubated in Warburg flasks at 33 C pH 7 3 for 20 min The temperature equilibrium period was 8 min The *main chamber* contained mito chondria (7 3 mg of protein from the patient 11 4 mg from the control) ADP (3 3 mM) potassium phosphate (10 mM) $MgCl_2$ (3 3 mM) *TES* buffer pH 7 3 (10 mM) and KCl (0 15 M) The volume was 3 ml The experiment was start d by the addition of substrate hexokinase (1 5 mg) and glucose (100 *μmoles*)

Substrate (μ moles)	Oxygen uptake (mμ atoms 0 mg of protein/ 0 min)	
	Pat	Contr
Pyruvate (10) malate (10)	490	840
Pyruvate (10)	510	570
Succinate (0)	1 060	5 0
Caprinyl-carnitine ( )	1 060	550

Liver mitochondria The respiration rate in isolated liver mitochondria with pyruvate a substrate is shown in Table 5 As reference sub trates were u ed succinate and caprinyl carnitine In the control mitochondria the oxy gen uptake was about half of that in mitochon dria from the patient In the patient the res piratory rate with succinate was in the same range as previously found with *human liver* mitochondria (14) Pyruvate (with or without malate) gave a respiratory rate which was *only* half the value obtained with the reference sub-

about 12% of the injected radioactivity was expired as CO_2 during the *in vivo* study

The radioactivity incorporated into glucose and liver glycogen was examined We were unable to demonstrate significant radioactivity present in the glucose, all being found as lactate and pyruvate In the tissues removed shortly after death the amount of radioactivity in liver glycogen corresponded to only about 0 3% of the dose injected 4 ½ hours previously

Table 3 shows the distribution of total radioactivity among various organs after death No excess activity was found in the liver and kidney, which normally have greatest capacity for gluconeogenesis showing that no metabolite has accumulated It appeared to be an even organ distribution of radioactivity with an average of about 2 500 cpm/g w w Since the body weight was 3 kg about 7.5×10^6 cpm of the injected radioactivity was retained i e 80% of the dose An elimination of 20% thus calculated compares well with the 13% measured (see above)

According to the estimated rate of serum lactate removal ($T/2=3$ hours) about 60% of the dose should have been metabolized during 4 hours Since the oxidation gluconeogenesis and urinary excretion can account for only 13%, the major part of the blood decay must therefore be due to a continuous diffusion of lactate into other body compartments By calculation of the relative specific activity of respiratory CO_2 and serum lactate (Fig 2) it was revealed that an equilibrium was not reached till 3 hours after the injection A delay due to the mixing of $^{14}CO_2$ formed with the body pool of CO_2/HCO_3^- can account for maximally 20 min of these 3 hours An equilibration period of 2 ½–3 hours does not appear to be strikingly long when compared with the equilibration time of about 2 hours found in adults with normal blood lactate (20) A normal permeability barrier for lactate in the cell membrane has in fact been demonstrated (28) in contrast to earlier concepts based on experiments with erythrocytes (15)

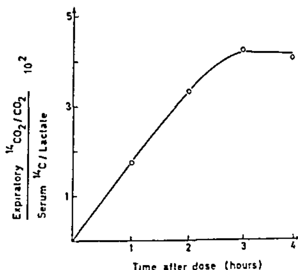


Fig 2 The ratio between the specific ^{14}C activity of respiratory CO_2 and serum lactate during the *in vivo* study

In accord with the above interpretation the decrease in blood radioactivity (Fig 1) mainly reflects the diffusion rate of lactate Also the metabolic removal of lactate can be estimated roughly from the *in vivo* study Lactate is assumed to equilibrate with total body water, i e in our patient about 2 100 ml representing 70% of the body weight Assuming an immediate and complete mixing of the injected radioactivity a serum radioactivity of 440 cpm/100 µl would be expected Using this value and the serum radioactivity after equilibration was reached (3 and 4 hours after dose) the decay curve due to metabolism can be drawn (stippled curve Fig 1) This gives a rate constant for the metabolism of lactate of about 0 058 per hour ($T/2 = 12$ hours) Under steady state conditions therefore about 18% of the radioactive lactate would have been removed in 4 ½ hours which compares well with the 13% measured (see above) With a body pool of lactate of 2 300 mg (distribution volume 2 100 ml serum concentration 108 mg/100 ml) the metabolic turnover of lactate is calculated to 44 mg/kg body weight/hour In normal adults Kreisberg et al (20) found a mean turnover rate of about 81 mg/kg body weight/hour (range 62–96)

Finally it appeared of interest to evaluate

Table 4 *The metabolism of lactate and pyruvate in liver and muscle slices*

Tissue slices (300-500 mg w w) were incubated in Ringer phosphate-saline solution at pH 7 4 for 1 hour at 37 The final volume was 3 5 ml The values are mean of two parallels The content of liver glycogen at the start of the experiment was 1 6 g/100 g in tissue from the patient and 5 6 in the control tissue

Substrate (µ moles)	Liver slices: CO_2 formed (nmoles/g w w) Pat	Contr	Incorporated into glycogen (nmoles/g w w) Pat	Contr	Glycogen content at the end of incubation (g/100 g w w) Pat	Contr	Muscle slices: CO_2 formed (nmoles/g w w) Pat	Contr
Lactate 1 C (10)	2 116	760	7	33	0 5	1 5	3 430	657
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the relative contribution of the oxidation to the total lactate metabolism From the values obtained for radioactive CO expiration when steady state condition was reached (Table 2) from the specific activity of lactate in the body and from the metabolic turnover rate for lactate it can be calculated that the oxidation to CO accounts for about 60 of total lactate removed

In vitro experiments

Tissue slices Table 4 shows that when liver slices obtained at partial autopsy were incubated with pyruvate and lactate significant amounts of $^{14}CO_2$ were produced The ^{14}CO formation was about twice as great with pyruvate as with lactate With both substrates however the ^{14}CO production was significantly higher in the slices from the patient than from the control Since lactate was equally efficient as substrate in the patient as in the control a defect in LDH was apparently not present Only small amounts of the radioactivity wa recovered in the liver glycogen at the end of the incubation about 5 times more in the slices from the control than from the patient In the liver slices from both infants the glycogen content was considerably reduced during the incubation showing that no net gluconeogenesis had occurred Thus as expected from the incubation conditions chosen (11) glycolysis and oxidation dominated over gluconeogenesis in the experiments with liver slices Muscle slice from the platient and the control showed a pattern of oxidation similar to liver slices when incubated with radioactive lactate and pyruvate (Table 4)

Liver mitochondria The respiration rate in isolated liver mitochondria with pyruvate a substrate is shown in Table 5 As reference sub trates were u ed succinate and caprinyl carnitine In the control mitochondria the oxygen uptake was about half of that in mitochondria from the patient In the patient the respiratory rate with succinate was in the same range as previously found with human liver mitochondria (14) Pyruvate (with or without malate) gave a respiratory rate which was only half the value obtained with the reference sub-

Table 5 *Oxidation of pyruvate by liver mitochondria*

Liver mitochondria were incubated in Warburg flasks at 33 C pH 7 3 for 20 min The temperature equilibrium period was 8 min The main chamber contained mitochondria (7 3 mg of protein from the patient 11 4 mg from the control) ADP (3 3 mM) potassium phosphate (10 mM) $MgCl_2$ (3 3 mM) TES-buffer pH 7 3 (10 mM) and KCl (0 15 M) The volume was 3 ml The experiment was started by the addition of substrate hexokinase (1 5 mg) and glucose (100 µmoles)

Substrate (µ moles)	Oxygen uptake (mµ atoms O mg of protein/20 min) Pat	Contr
Pyruvate (10) malate (10)	490	840
Pyruvate (10)	510	570
Succinate (20)	1 060	570
Caprinyl-carnitine (?)	1 060	550

about 12% of the injected radioactivity was expired as CO_2 during the *in vivo* study

The radioactivity incorporated into glucose and liver glycogen was examined. We were unable to demonstrate significant radioactivity present in the glucose, all being found as lactate and pyruvate. In the tissues removed shortly after death, the amount of radioactivity in liver glycogen corresponded to only about 0.3% of the dose injected 4 1/2 hours previously.

Table 3 shows the distribution of total radioactivity among various organs after death. No excess activity was found in the liver and kidney which normally have greatest capacity for gluconeogenesis, showing that no metabolite has accumulated. It appeared to be an even organ distribution of radioactivity with an average of about 2 500 cpm/g w.w. Since the body weight was 3 kg, about 7.5×10^6 cpm of the injected radioactivity was retained, i.e. 80% of the dose. An elimination of 20% thus calculated compares well with the 13% measured (see above).

According to the estimated rate of serum lactate removal ($T/2 = 3$ hours) about 60% of the dose should have been metabolized during 4 hours. Since the oxidation, gluconeogenesis and urinary excretion can account for only 13%, the major part of the blood decay must therefore be due to a continuous diffusion of lactate into other body compartments. By calculation of the relative specific activity of respiratory CO_2 and serum lactate (Fig. 2) it was revealed that an equilibrium was not reached till 3 hours after the injection. A delay due to the mixing of $^{14}CO_2$ formed with the body pool of CO_2/HCO_3^- can account for maximally 20 min of these 3 hours. An equilibration period of 2 1/2–3 hours does not appear to be strikingly long when compared with the equilibration time of about 2 hours found in adults with normal blood lactate (20). A normal permeability barrier for lactate in the cell membrane has in fact been demonstrated (28) in contrast to earlier concepts based on experiments with erythrocytes (15).

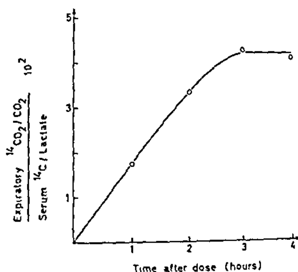


Fig. 2. The ratio between the specific ^{14}C activity of respiratory CO_2 and serum lactate during the *in vivo* study.

In accord with the above interpretation the decrease in blood radioactivity (Fig. 1) mainly reflects the diffusion rate of lactate. Also the metabolic removal of lactate can be estimated roughly from the *in vivo* study. Lactate is assumed to equilibrate with total body water, i.e. in our patient about 2 100 ml representing 70% of the body weight. Assuming an immediate and complete mixing of the injected radioactivity, a serum radioactivity of 440 cpm/100 µl would be expected. Using this value and the serum radioactivity after equilibration was reached (3 and 4 hours after dose) the decay curve due to metabolism can be drawn (stippled curve Fig. 1). This gives a rate constant for the metabolism of lactate of about 0.058 per hour ($T/2 = 12$ hours). Under steady state conditions therefore about 18% of the radioactive lactate would have been removed in 4 1/2 hours which compares well with the 13% measured (see above). With a body pool of lactate of 2 300 mg (distribution volume 2 100 ml, serum concentration 108 mg/100 ml) the metabolic turnover of lactate is calculated to 44 mg/kg body weight/hour. In normal adults Kreisberg et al. (20) found a mean turnover rate of about 81 mg/kg body weight/hour (range 62–96).

Finally it appeared of interest to evaluate

creased production has also been proposed by Erickson (8) in another patient on the basis of lactate determinations in both venous and arterial blood after muscular exercise Israels et al (17) and Scharer et al (29) on the other hand demonstrated an increased urinary excretion of α ketoglutarate in the former study upon administration of glutamic acid These findings were interpreted to indicate a defect in the citric acid cycle resulting in a decreased oxidation of lactate (12 17 29) A third possibility appears to us to be a block in the gluconeogenesis That an inhibition of this pathway in fact leads to lactate accumulation is shown in animal experiments using tryptophan as an inhibitor of the gluconeogenesis (26)

Our patient had been in steady state regarding lactate metabolism for several days before the *in vivo* study The removal of lactate from the body must therefore equal the formation and the turnover rate was estimated to 44 mg/kg/hour This is lower than that recently found in normal adults (62–96 mg/kg/hour) (20) This allows us to exclude an abnormally high production of lactate as the cause of the hyperlactacidemia Consequently a reduced capacity of lactate removal must exist Lange Andersen et al (2) and Knuttgen (19) showed that after exercise the blood lactate in adults decreases exponentially with a $T/2$ of 25 min The rate of lactate removal in our patient was estimated to equal a halftime of 12 hours If $T/2$ had been 25 min instead of 12 hours in our patient the disappearance rate would have been 1 300 mg kg hour (calculated from a pool of 2 300 mg) as compared with the 44 mg/kg/hour actually found In normal children (12) and adults (6) infusions of lactate without raising serum lactate above normal have shown a removal capacity of 150–210 mg/kg hour which is considerably above the normal demand When compared with previous results therefore the present study clearly shows an abnormally low capacity for lactate removal in our patient.

As discussed above one possible cause of the delayed removal of lactate might be a reduced capacity for its oxidation The *in vivo* study showed that oxidation accounted for about 60% of the estimated lactate removal In comparison Kreisberg et al (20) in adults could account for 11% of the lactate removal by oxidation From the present *in vivo* study it may be concluded that oxidation contributes relatively much to the lactate removal in our patient In addition the *in vitro* experiments with tissue slices as well as mitochondria did not reveal an insufficient capacity for the oxidation of lactate/pyruvate

The other possible way of lactate removal is by gluconeogenesis Since the oxidative pathway appeared to function normally one will *a priori* suspect a block in the gluconeogenetic pathway Several of the present observations support this concept 1) A very low glycogen content was found both in liver and muscle 2) Only trace amounts of radioactive glycogen in liver were found $4\,^1/_2$ hours after the injection of ^{14}C lactate 3) No measurable amount of radioactivity could be traced in blood glucose during $4\,^1/_2$ hours after the injection of ^{14}C lactate In contrast de Meutter & Shreeve (22) found that 10–15 of ^{14}C lactate when injected in humans was recovered in the extracellular glucose during the following 2 hours Kreisberg et al (2) showed that gluconeogenesis accounted for at least 21% of total lactate removal in normal adults 4) The ratio between the specific radioactivity of respiratory CO and blood lactate upon ^{14}C lactate injection reached a steady level in 3 hours (Fig 2) suggesting that a recycling of radioactivity from glucose did not occur Kreisberg et al (20) found in adults with normal gluconeogenesis a steady increase in this ratio for more than 4 hours

Since 15 to 20% of the blood glucose normally is derived from lactate (20 27) hypoglycemia might be expected when a defect in the gluconeogenesis is present. However our patient received almost continuously parenteral nutrition with glucose-containing solutions from the second day of life making hypoglycemia unlikely to develop Based on the above

strates in mitochondria from the patient whereas pyruvate + malate gave a maximal respiratory rate in the control Since pyruvate was oxidized at the same rate even without the addition of malate as sparker, a deficiency of pyruvate carboxylase was apparently not present in the patient

LDH-isoenzymes Very high serum levels of lactate dehydrogenase (LDH) were found in our patient (21) Moreover, the LDH izoenzyme pattern in serum appeared abnormal The level of LDH_4 was high when compared with values from normal children aged 4–5 years (13) Further, this fraction was not detected in the control patient (Table 6)

The similarity of the patterns found in serum and liver suggests that the elevated LDH levels in serum is mainly caused by a release from the liver This conclusion is consistent with other signs of liver damage (21) LDH_5 could not be detected in any of the tissues from the patient The patterns in the tissues from the control appeared more normal (31, 32) The lack of LDH_5 is probably an unspecific sign since a shift in the LDH isoenzymes towards a relative decrease of LDH_5 is regularly seen in fetal tissues (7 18) on denervation (5) and in different neuromuscular diseases (31)

Table 6 *Isoenzymes of lactate dehydrogenase*

The activity of each LDH fraction is expressed as of the total activity as estimated by scanning electropherograms in agarose gel The values of the control patient are given in parentheses

	LDH fraction				
	1	2	3	4	5
Serum	28 (30)	28 (40)	33 (30)	11 (0)	0 (0)
Muscle (ileo psoas)	25 (13)	47 (14)	28 (23)	0 (30)	0 (30)
Liver	13 (4)	23 (7)	25 (2)	39 (43)	0 (43)
Kidney	48	35	17	0	0

Table 7 *Glycogen content of liver and muscle*

The values (mean of two parallels) are g/100 g tissue w w

	Liver	Muscle
Patient	1 6	<0 01
Control	5 6	0 5

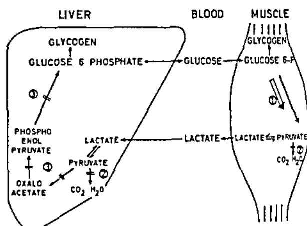


Fig 3 Schematic illustration of lactate metabolism with indication of possible defective pathways in congenital lactic acidosis *1* increased glycolysis *2* decreased oxidation *3* decreased gluconeogenesis

Glycogen content Table 7 shows that the glycogen was reduced in the liver and not measurable in the muscle of the patient In the control the glycogen content of the liver was in the lower normal range, whereas in muscl it was somewhat decreased as compared to that found in biopsy material from normal adults (16) The low values in the control may be ascribed to agonal changes

DISCUSSION

The present patient like several previously reported (4 8 10, 12 17 24, 25, 29 33) demonstrated hyperlactacidemia of unknown reason as a cardinal sign The existence of a metabolic error some place in the Cori cycle (Fig 3) or in the oxidative pathway may be anticipated The accumulation of lactate could be due to a specific enzyme defect but could also be secondary to a toxic effect of an accumulated metabolite in analogy with the accumulation of blood glycine in methylmalonic acidemia (9) Worsley et al (33) on the basis of erythrocyte experiments suggested that an increased lactate production may be the cause of the hyperlactacidemia of their patient An in

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findings therefore, the conclusion seems warranted that a block in the gluconeogenesis was present in our patient

Previous studies in children with hyperlactacidemia have suggested a functioning gluconeogenesis Thus Erickson (8) found a drop in the blood lactate level across the liver in 2 cases Haworth et al (12) observed an increase in the blood glucose levels during infusion of lactate to patients with hyperlactacidemia The discrepancy between these results and the present findings suggests that congenital lactic acidosis might be a heterogenous group of diseases

SUMMARY

Biochemical studies *in vivo* and *in vitro* were performed in a newborn child suffering from congenital lactic acidosis A block in the oxidation of lactate as well as an abnormally high rate of glycolysis have previously been suggested to be of etiological significance In the present studies direct evidence has been obtained for a decreased capacity for gluconeogenesis as the cause of the lactate accumulation In addition the rate of glycolysis was not increased and no block in the oxidation of lactate was present

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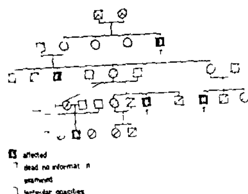


Fig 1 Pedigree of the family Within each generation individual members are numbered from left to right The proband i marked by an arrow

II 5 (V H A) male infant born in 1914 He was delivered at home 3 weeks prematurely B cause of poor weight gain he was admitted to a pediatric ward when he was 2½ months old According to the clinical record his head was enlarged and he was unable to follow objects with his eyes Bilateral cataract was found He was hypotonic and showed signs of mild rickets The presence of proteinuria was also demonstrated (3 g per liter) At the age of 9 months he was completely blind and was transferred to an infants home where he soon died from pneumonia

2 *Possible cases of the Lowe syndrome*

III 1 male infant born in 1925 died at the age of 9 months presumably from an infectious disease Ocular abnormalities were not observed

III 2 male infant born in 1926 died shortly after birth Information about the eyes is not available

V 1 male infant (1964) stillborn macerated Information about the eyes is not available

3 *Carriers*

IV 3 born in 1945 healthy On ophthalmologic examination the visual acuity in both eyes was 6 6+0 50 cyl 90 In both lenses were seen peripheral coerulea elements as well as delicate opacities at the posterior pole resembling a congenital posterior polar cataract

III 5 born in 19 8 healthy Ophthalmologic examination showed visual acuity 6 6+0 50 sph In both lenses a delicate punctuated cataract was confined largely to the embryonic core

III 7 born in 1931 healthy In 1959 she was examined by Seedorff (7) and by Tersley (9) who found punctate opacities in both lenses. On the present ophthalmologic examination visual acuity was 6 6+1 00 sph in both eyes Both lenses showed incipient cataract Minute discrete opacities were scattered throughout the lens somewhat larger rounded opacities were located at the periphery of the lens.

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The following female members of the family have a 50% risk of being carriers

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Three fathers of affected children were also examined All had normal eyes without evidence of cataract The proband s father was not examined

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Urins from three male members (II 1 III 8 and IV 11) as well as from four female member of the family (III 7 IV 3 IV 6 and IV 12) were examined for amino acids by means of paper chromatography In all cases normal rates of excretion were observed

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Seven members of the family including the proband were examined for Xg blood group antigens [1] Unfortunately all of these proved to be group Xg (a+) Hence no information about the relative locations of the Lowe gene and the Xg gene on the X chromosome was obtained

DISCUSSION

All affected boys in this family showed the features typical of the oculo-cerebro-renal syndrome Transmission was sexlinked the complete syndrome occurring in boys only However Scholten (6) and Svorc et al (8) have described sporadic cases in girls Thus Svorc et al reported a girl with bilateral congenital

[1] Kindly performed by Ruth Sanger Medical Research Council Blood Group Unit, Lister Institute London

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THE OCULO CEREBRO RENAL SYNDROME OF LOWE IN FOUR GENERATIONS OF ONE FAMILY

G PALLISGAARD and E GOLDSCHMIDT

From the Department of Paediatrics and Department of Ophthalmology Odense University Hospital Odense Denmark

Following the original description in 1952, by Lowe and associates (4) a number of reports on the oculo cerebro-renal syndrome have appeared and in 1968 Abassi et al (1) published an extensive review of its clinical aspects, adding several cases of their own The Lowe syndrome is a congenital disease in boys characterized by ocular abnormalities, including cataract and/or glaucoma severe psychomotor retardation, muscular hypotonia, and renal manifestations comprising renal tubular acidosis with rickets, aminoaciduria, and proteinuria

Whereas the clinical features are thus fairly conspicuous, the genetic aspects of this syndrome remain largely unelucidated We have had the opportunity to investigate a family in which the Lowe syndrome has been transmitted through four generations This report presents the information gathered with particular reference to the genetic analysis

DESCRIPTION OF THE FAMILY

Part of the pedigree of this Danish family comprising the four affected generations are shown in Fig 1 Results of examinations of individual family members are presented in the following

1 *Proven or probable cases of the Lowe syndrome*

V 3 (A C) the proband male infant born in May 1967 Immediately following delivery cloudiness of the infant s pupils was noted as were marked muscular hypotonia and retention of the testes Ophthalmologic examination revealed bilateral cataract At the age of 3½ months elevated intraocular pressures were recorded the corneas being cloudy and oedematous

Several goniotomias were performed but deterioration of the boy s general condition precluded a proper therapeutic regimen and amaurosis supervened His psychomotor development has been severely retarded and at 20 months of age his developmental age was estimated to be about 3 months and further progress has not been achieved Renal tubular acidosis required treatment from the age of 4 months and was followed shortly by the appearance of rachitic bone changes At the age of 7 months the patient was treated surgically for a left ureteral stricture Intermittent glucosuria was noted through the first 3 months of life only whereas mild proteinuria has persisted A selective amino-aciduria has been a constant finding Serum creatinine and urea concentrations have remained within normal limits A chromosomal analysis was normal Further details of the findings in this patient are reported elsewhere (3)

IV 8 (C O K) boy born in 1961 delivered by Caesarean section because of fetal bradycardia He was asphyxiated at birth and was given oxygen Shortly following delivery bilateral buphthalmos was diagnosed the corneas being enlarged and cloudy Bilateral cataract was also present The infant thrived poorly and died 4 weeks old At autopsy a large hematoma of the liver and partial atelectasis of the right lung were the only abnormal findings *The eyes* were not examined

IV 10 (B C) boy born in 1957 Glaucoma and cataract were present at birth Psychomotor development was retarded and hearing impaired Additional findings included amino-aciduria proteinuria and mild metabolic acidosis He died from aseptic meningo-encephalitis at age of 1½ years Further details of this case have been reported by Seedorff (7) and by Terslev (9)

III 3 (V C) boy born in 1927 According to information provided by his father the infant was born at home blind with enlarged eye balls and later developed rickets Shown a photograph of the proband the father confirmed a close resemblance The boy died from pneumonia at the age of 8 months without being admitted to hospital

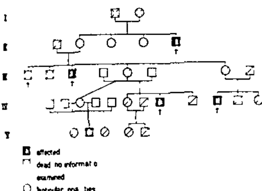


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V 3 (A C) the proband male infant born in *May* 1967 Immediately following delivery cloudiness of the infant s pupils was noted as were marked muscular hypotonia and retention of the testes Ophthalmologic examination revealed bilateral cataract At the age of 3½ months elevated intraocular pressures were re corded the corneas being cloudy and oedematous

Several goniotomias were performed but deterior tion of the boy s general condition precluded a prop therapeutic regimen and amaurosis supervened H psychomotor development has been severely retard and at 20 months of age his developmental age w estimated to be about 3 months and further progre has not been achieved Renal tubular acidosis requir treatment from the age of 4 months and was followe shortly by the appearance of rachitic bone chang At the age of 7 months the patient was treat surgically for a left ureteral stricture Intermitte glucosuria was noted through the first 3 months life only whereas mild proteinuria has persisted selective amino-aciduria has been a constant findi Serum creatinine and urea concentrations have r mained within normal limits A chromosomal analy was normal Further details of the findings in t patient are reported elsewhere (3)

IV 8 (C O K) boy born in 1961 delivered Caesarean section because of fetal bradycardia I was asphyxiated at birth and was given oxygen Sho ly following delivery bilateral buphthalmos was di nosed the corneas being enlarged and cloudy lateral cataract was also present The infant thriv poorly and died 4 weeks old At autopsy a lar hematoma of the liver and partial atelectasis of t right lung were the only abnormal findings The ey were not examined

IV 10 (B C) boy born in 1957 Glaucoma an cataract were present at birth Psychomotor develop ment was retarded and hearing impaired Addition findings included amino aciduria proteinuria and mil metabolic acidosis He died from aseptic meningo-en cephalitis at age of 1½ years Further details of th case have been reported by Seedorff (7) and by Te slev (9)

III 3 (V C) boy born in 1927 According to in formation provided by his father the infant was bor at home blind with enlarged eye balls and late developed rickets Shown a photograph of the pro band the father confirmed a close resemblance Th boy died from pneumonia at the age of 8 mont without being admitted to hospital

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DISTRIBUTION OF PULMONARY BLOOD FLOW IN CHILDREN WITH CYSTIC FIBROSIS

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Pulmonary involvement is responsible for most of the complications and mortality in cystic fibrosis Since plugging of airways with excessive secretion of mucus is one of the main features of pulmonary pathology it might be expected that besides close clinical follow up ventilatory function studies should be most appropriate in assessment of the severity of pulmonary involvement (4 8 9 12 21) Unfortunately in childhood during several critical years spirometric measurements are difficult or impossible for technical reasons Moreover these techniques are not able to detect regional differences in lung function Therefore other methods must have been searched for

Lung scanning has been extensively used for the evaluation of pulmonary blood flow distribution in different clinical conditions An attempt to use the measurement of pulmonary blood flow distribution for the assessment of disturbances primarily in ventilation was stimulated by our previous work on ventilation–perfusion relationships (13) The advantage of this method for the studies of regional pulmonary function in children with the obstruction of the airways including cystic fibrosis has been documented (6 14) The present report concerns the regional changes in distribution of pulmonary blood flow and their value for the early detection and follow up of pulmonary function abnormalities in cystic fibrosis Special attention was paid to repeated measurements of pulmonary blood flow distribution in the same patient

MATERIAL AND METHODS

Thirty lung scans were obtained in 19 children below 6 years of age and in . older patients with cystic fibrosis (Table 1) The diagnosis was based on the clinical history and on repeated physical and radiological examinations over a long period It was confirmed by analysis of the sweat chloride concentration using the method of electrical conductivity (3) Criteria of Shwachman & Kulczycki (17) were used to score the clinical condition of our patients Body height and weight were expressed in percentiles (18) Chest roentgenograms were obtained by the usual technique in the upright position with the exception of children younger than 1 year of age in which the supine position was preferred The pictures were taken in the maximal inspiration Respiratory function studies have been performed only in 5 children (cases . 4 15 16 18) because of the low age of most of our patients and they are included in the previous report (7) Arterialized capillary blood oxygen tension was measured by the microelektrode of Clark type The acid base status of the blood was determined at 37 C by the Astrup equilibration technique ( ) Regional blood flow distribution was measured by lung scanning Macroaggregated human serum albumin (particle size 10 to 75μ) tagged with ^{99m}Tc was injected intravenously in the same position as used for taking X ray pictures The isotope dose ranged between 50 and 500 microcuries according to the age of the patient The total amount of albumin did not reach 0 001 mg kg body weight A

cataract, mental retardation and proteinuria A sex chromatin study and a chromosomal analysis showed female characteristics The occurrence of the syndrome in girls must be assumed to represent manifest heterozygotism in accordance with the Lyon hypothesis

The findings in carriers are in agreement with the rather limited evidence on record In the present family, all carriers had lenticular opacities However, the lenticular changes varied, coerulea elements, peripheral spokes and posterior polar cataract being found within the family Wilson et al (10) likewise observed polar cataract in one carrier Abassi et al (1) did not find lenticular changes in all mothers of affected children On the contrary, in one family studied by them, the father of an affected boy had lenticular opacities and similar observations in fathers have been reported by Richards et al (5) Illig et al (2), and Wilson et al (10) However, slight lenticular changes occur frequently and may be observed in 25% of all persons from the age of 40 years Hence the fact that lenticular opacities are found in some fathers does not necessarily mean that a particular parental configuration is required in order to produce the complete syndrome The cataracts found in the eyes of carriers correspond well to those which may be observed in an appreciable fraction of an ophthalmologic clientele However in the family studied by us, the changes seem to appear early, and in one conductor (II 2), operations were required before the age of 50 years Even though another carrier (III-7) still has a normal vision, the incipient cataract will probably soon cause visual disturbances Although lenticular changes may not occur in all carriers we find it advisable, prior to genetic counselling to perform an ophthalmologic examination on possible carriers of the Lowe syndrome

SUMMARY

A Danish family with five proven and three additional possible cases of the oculo cerebrorenal syndrome is described All proven carriers had cataract, but the lenticular opacities varied considerably with respect to magnitude appearance, and location In this family, the disease was transmitted as a sex linked recessive trait, the complete syndrome being seen in boys only

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Key words Oculo cerebro renal syndrome Lowe's syndrome genetics mode of inheritance

Chest roentgenogram				Lung scan Underperfusion	
Peribronchial thickening					
Grade	Localization	Localized density	Hyperaeration Grade	Grade	Localization
0	—	0	0	0	—
2	RUt RLt	0	1 d	3	RUp RMp
1	LLp			2	RUt
1	RUt LUt	RUt	1 d	0	—
1	RUt LUt	RUt	1 d	2	RUt
				1	RLp
2	RMp RLp LLp	0	1 d	3	LUt
				2	RMp
2	RMt RLt LLp	0	2 d	2	LUt RMp
1	RUt RLp LUt	0	0	0	—
1	RUp RLp	0	0	0	—
1	RUt RMt LUt LMt	0	2 d	1	RUt
2	RUp RLp LUp LLp	0	1 d	0	—
2	RUt RMt LUt	0	1 d	1	Multipl
3	Generalized	0	2 d	3	RUp RMp LUt
				2	RUt RLp
1	RLp LLp	RLp	2 d	3	RUp RMt
1	RUt LUt	RLp	3 d	2	RLp LUp
1	RUp RLp	0	1 d	1	LUp
2	RLp LMp	RLp LMp	1 d	0	—
2	RUt RMt RLt LMt	LMp RUp	2 d	0	—
3	RMt RLt LMt LLt	RMp LMp	3 c	2	RUp LUp
3	RUt RMt RLt LMp LLt	LLp	2 d	2	RUp RMp RLp LLp
3	RMt RLt	0	3 d	2	RUp RMp RLp LLp
				3	RUt LUt
3	RMt RLt	0	3 d	2	RMp LMp
				3	RUt LUt
3	RMt RLt	0	3 d	2	RMp LMp
				3	RUt LUt
3	Generalized	RLp RMp LLp	2 c	2	RMp LMp
				3	RUt
3	Generalized	0	3 d	2	LUp LLp
				3	RUt RMp LUp
3	Generalized	0	3 d	2	LMp
				3	RUt RMp
3	Generalized	0	3 c	2	LMp
				2	RUp RMp RLp LLp
2	RUp RMp LUp LMp	RUp LLp	2 d	2	RUp RMp LUt LMp
2	RUt RLp LLp	0	1 d	0	—
2	RUp LUp	RLp	1 d	0	—
				2	RUt

series (Table 1) The most frequent incidence of local changes in perfusion was encountered in the right upper area followed by the right middle and left upper area (Table 2 and Fig. 1) The combination of partial and total involvement of several areas was usual Only in 4 children solitary total or partial underperfusion of one area appeared Marked and moderate changes in perfusion or their combination were most frequent

By dividing our patients according to the

Table 2 *Regional distribution of underperfusion*

Lung area	Incidence of underperfusion (%)
Right	
Upper	81
Middle	67
Lower	29
Left	
Upper	57
Middle	29
Lower	33

Table 1 *Data on patients and results of studies*[a]

Case no	Name	Sex	Age (y mo)	Height (per centil)	Weight (per centil)	Sweat Cl (mEq/l)	Clinical score	Arterial blood			
								P_{O_2} torr	P_{CO_2} torr	pH	HCO_3 (mEq/l)
1	P T	♂	3 3	70	50	96 6	85	82	31	7 362	17 1
2	R D	♀	5 9	50	70	102 4	85	73	39	7 386	23 0
3	M H	♀	0 6	40	50	114 4	78	85	36	7 406	[illegible]
			0 7	40	50	—	78	—	—	—	—
4	M V	♀	11 7	20	30	100 4	78	102	37	7 450	25 1
			11 8	20	30	—	78	90	34	7 455	23 5
5	M M	o	1 5	90	50	103 9	77	90	31	7 419	19 4
6	E J	♂	4 9	10	70	81 7	76	112	28	7 470	17 8
7	A H	o	3 6	10	20	128 4	73	—	31	7 440	[illegible]
8	J P	♀	4 9	10	40	104 4	68	—	—	—	—
9	P H	♂	3 10	10	3	124 4	67	103	38	7 456	25 0
10	T L	♂	5 0	3	20	115 4	65	70	37	7 398	22 0
11	P M	♂	1 1	10	10	108 9	64	70	37	7 400	22 3
			2 2	30	50	—	63	68	37	7 371	20 7
12	H P	♀	3 6	10	3	89 2	63	93	39	7 385	23 0
13	L B	♀	1 8	3	3	116 9	63	90	39	7 412	24 5
14	M S	♂	0 5	70	10	82 6	55	75	49	7 373	28 1
15	M H	♂	3 0	80	20	117 4	55	—	48	7 343	25 3
			4 7	20	30	—	49	76	51	7 355	28 1
			4 8	20	30	—	—	—	45	7 409	28 0
16	L S	♂	20 2	90	40	70 2	54	—	—	—	—
			20 3	90	40	—	54	53	41	7 432	26 9
			20 4	90	40	—	54	55	40	7 476	25 9
17	D F	♀	4 4	3	10	84 4	51	58	42	7 393	24 8
18	J H	♂	5 11	3	3	120 5	50	100	34	7 442	[illegible]
			6 11	5	3	—	46	70	33	7 406	20 3
19	K M	♀	4 5	30	3	115 4	50	80	40	7 458	27 0
			4 6	30	3	—	45	80	40	7 439	26 1
20	L M	♀	2 1	3	3	101 3	49	77	44	7 388	25 6
			3 0	3	3	—	52	84	34	7 399	20 2
21	A D	♀	1 0	10	3	91 9	48	83	29	7 448	19 2

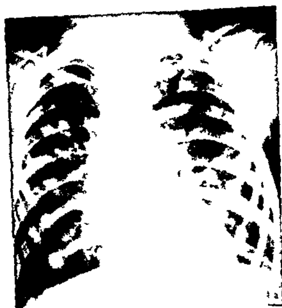
[a] Grade 0 = normal 1 = slight 2 = moderate 3 = marked Localization RU RM RL = right upper middle lower area LU LM LL = left upper middle lower area t = total p = partial d = diffuse c = cystoid

rectilinear scanner (Pho/Dot Nuclear Chicago) equipped with 127 hole collimator was used for the detection of the distribution of the macroaggregates Both X ray picture and lung scan were usually taken within the shortest possible period of time in the same day

The evaluation of *regional perfusion* was done in a similar way as in the chest roentgenograms Both lungs were divided by the horizontal lines into three even areas not corresponding exactly to the lung lobes Peribronchial thickening hyperaeration on roentgenograms and underperfusion on lung scan was divided according to the intensity into 3 grades The radioactivity equal to the background activity is called underperfusion of the grade 3 in contrast to the underperfusion of the grade 0 equal to the maximal radioactivity concentration Partial or total involvement of individual areas indicates the extent of these changes In 8 patients repeated measurements of regional pulmonary blood flow were performed with an interval ranging from 1 week up to 1 year and 8 months

RESULTS

Normal distribution of the pulmonary blood flow was detected only in 8 children in our



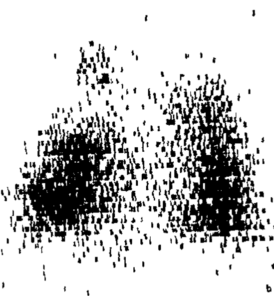


Fig 3 T L (case 10) (a) Chest roentgenogram (b) Lung scan Multiple defects in perfusion contrasted with the generalized image on chest roentgenogram

Both normal and pathological local distribution of pulmonary blood flow was detected in children with the impairment of pulmonary gas exchange indicated by the elevation of P_{CO} and by the decrease in P_O values in arterialised capillary blood Correlation between the severity of distribution abnormalities and disturbances in gas exchange was not revealed

Rad ological evidence of pulmonary involve m nt participates in clinical scoring However comparison of lung scans with chest roentgenograms alone was necessary for the estimation of th value of both techniques in patients with cystic fibrosis (Table 1) In all but one patient peribronchial thickening of different intensity was evident from the chest roentgenogram In the majority of ca es localization of radiological changes and abnormalities in the distribution of radioactivity correlated only partially In one third of investigations marked discrepancy was found Focal changes in density were d tected on 11 roentgenograms Their localization did not agree in 64% of investigations with the localization of perfusion inequalities On the other hand areas of marked underperfusion were revealed by lung scanning in patients without focal changes in X ray density (Fig 3)

Repeated canning was performed in 8 patients Different pictures of blood flow distribution were obtained in 5 patients (case 3 11 15 *a* 18 19) Marked changes in the distribution pattern within an interval shorter than 1 month were revealed in two of them without apparent change of the clinical condition (case 3 11) In the third child (case 15) the spreading of distribution abnormalities after a longer interval was accompanied by the d terioration of the clinical condition The change only in the localization of underperfused areas was combined with the lowering of clinical score in two children (case 18 19)

Unchanged distribution was found three times (case 4 16 20) after an interval of 1 month or 1 year respectively The discrepancy between stationary pattern of blood flow distribution and changing clinical picture was detected in two patients (case 16 20) During the first measurement one of them (case 20) was in a fair clinical condition Surprisingly lung scan showed marked disturbances in per



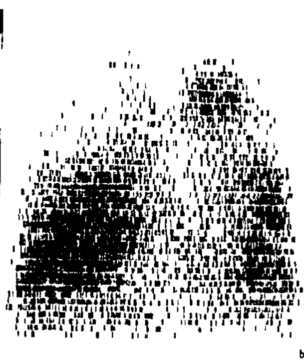
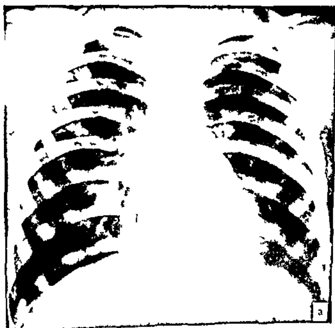


Fig 1 J H (case 18) (a) Chest roentgenogram shows generalized marked peribronchial thickening and diffuse hyperaeration (b) Lung scan shows most frequent localization of perfusion defects (see Table 1)

clinical scoring method (17) three groups became apparent Only in 50% of the patients in good clinical condition (case 1–7) normal pulmonary blood flow distribution was revealed (Fig 2) High frequency of abnormalities in distribution was encountered in the group with mild clinical condition (case 8–13) and in the group with the lowest clinical score (case 14–21) Nevertheless, in the latter group normal distribution pattern was also detected



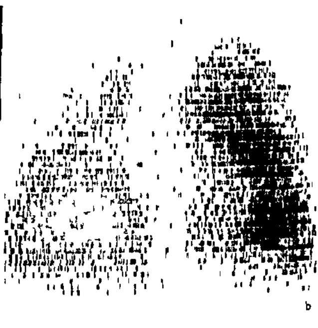


Fig 2 R D (case 2) (a) Chest roentgenogram shows moderate degree of peribronchial thickening mostly of the right side with diffuse hyperinflation of low degree (b) Lung scan demonstrate abnormalities in blood flow distribution not predictable either from clinical or radiological examination

local increase in pulmonary vascular resistance and consequently decrease of blood flow in this region (1)

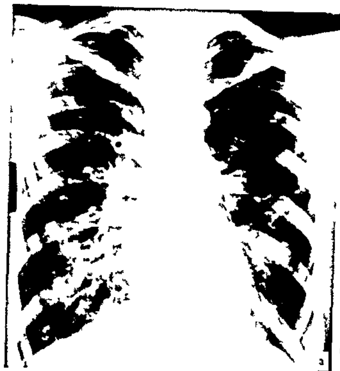
This mechanism was proved to be responsible for the perfusion abnormalities under several conditions leading to the local obstruction of the airways In acute asthmatic attack marked local defects in perfusion were demonstrated in children with otherwise normal pulmonary blood flow distribution (10 16) To prove that the obstruction of airways is responsible for the change on lung scan blood flow distribution was measured after partial bronchial obstruction by unilateral bronchography Transient perfusion abnormalities were *of th same character* as during an asthmatic attack (16) The changes in regional perfusion following localized obstruction of the airways were not identical with the changes in regional ventilation (19) On the contrary Gyepes et al (6) were able to demonstrate the local coincidence of ventilation and perfusion defects The existence of perfusion disturbances and their variability in our patients favours the participation of mechanism regulating perfusion according to the ventilation in pulmonary pathophysiology of cystic fibrosis

Open question remains the efficiency of this mechanism in maintaining perfect gas exchange If the diminution of perfusion matches localy and qualitatively the decrease in ventilation no disturbance in pulmonary gas exchange would develop However the studies of local regulation of ventilation perfusion ratio have shown some limitations of this mechanism (15 20) This may explain low ventilation perfusion ratio revealed in patients with cys c fibrosis (11) Whatever is the efficiency of this mechanism local inequalities of perfusion in our patients with the obstructive lung disease must have favourable effect on gas exchange If the lungs were not able to shift blood away from underventilated regions the incidence of abnormal values of arterial gas tension might be expected to be high r

Roentgenography has been widely used in the assessment of pulmonary involvement in cystic fibrosis The limitations of this method in detection of local changes in perfusion has been demonstrated by comparison with lung scanning in this study The lack of correlation between both methods was encountered The most frequent finding on chest roentgenograms was peribronchial thickening and diffuse hyperaeration There is no reason to expect changes of this character to be reflected by the local abnormality of perfusion Localized changes of X ray density without regard of their etiology should manifest themselves as the change in perfusion This was not the case in the majority of our patients On the other hand even marked defects in perfusion shown by lung scanning were not revealed by roentgenography In view of these facts there is no reason to underestimate the value of roentgenography in cystic fibrosis Its importance is primarily in depicting anatomical pulmonary le ions and its value was documented also in our study The main advantage of lung scanning is in the detection of regional changes in perfusion i e in the detection of a functional abnormality Our study has shown that both methods are very useful but not interchangeable

By use of criteria indicated above three clinical groups of patients were differentiated in our material The explanation of the lack of exact correlation between the severity of the illness and incidence of the disturbance in perfusion must be searched for in the above mention d mechanism of development of the perfusion changes If we accept the assumption that the regional abnormalities in perfusion are secondary to the ventilation disturbances then the d fects in perfusion depend solely on the magnitude and character of the airway obstruction On the other hand the clinical picture is composed also by many other factors

Variability in bronchial obstruction is typical for the early stage of pulmonary involvement whereas fixed changes can be interpreted as permanent disturbance of pulmonary function signalizing the unfavourable *progression* of the disease (8) Early detection of dynamics





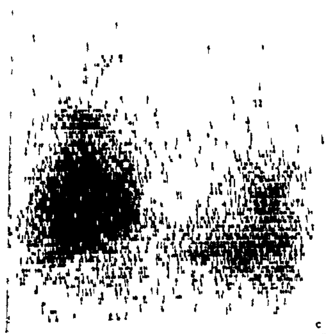


Fig 4 I S (case 16) (a) Chest roentgenogram taken at the same data as the second lung scan shows marked peribronchial thickening and hyperaeration with decreased density in left upper area (b) and (c) Lung scans recorded with an interval of 10 weeks show identical pattern of blood flow distribution

fusion (Fig 4 b) These changes did not improve after the intensive treatment One week later this patient was admitted to the hospital in respiratory distress due to acute respiratory infection and a small localized left sided pneumothorax After the recovery an identical distribution pattern persisted (Fig 4 c)

DISCUSSION

Lung scanning is believed to be very useful in the diagnosis of pathologic processes involving the pulmonary vascular bed However no significant changes in pulmonary arteries and arterioles were found in cystic fibrosis (5) The high incidence of the abnormalities in pulmonary blood flow distribution proved in our patients must therefore be caused by another mechanism This mechanism has not been firmly established yet It is reasonable to assume that disturbances in the patency of the airways play an important role in evoking secondary changes in perfusion by two principal mechanisms The obstruction of bronchi and/or bronchioles is followed by profound changes in the composition of alveolar gas namely decrease in P_{O} and increase in P_{CO} Increase in the airway resistance causes the enhanced respiratory fluctuations of transpulmonary pressure During expiration alveolar pressure distal to the partial airway obstruction increase considerably (9) Both changes in alveolar gas tension and in intraalveolar pressure elicite

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of these functional abnormalities by repeated lung scanning may have considerable implications in the management especially in the age when lung function studies are not available Repeated scanning can be used also as one of the indicators of the therapeutical effect Lung scanning as a highly reproducible simple and relatively safe procedure, available in any age offers new possibilities in clinical follow up of patients with cystic fibrosis and it should be included among other criteria for the assessment of clinical condition and mainly pulmonary involvement in cystic fibrosis

SUMMARY

Marked abnormalities of pulmonary blood flow distribution were detected in 13 out of 21 children with cystic fibrosis using lung scanning Underperfusion of different grade was localized most frequently in the right upper area followed by the right middle and left upper areas Correlation between clinical condition or impairment of gas exchange and degree of perfusion disturbances was lacking Abnormal chest roentgenograms were encountered more frequently than the definite disturbances in the distribution of perfusion On the other hand even profound defects of perfusion were not revealed by chest roentgenography Repeated lung scanning might be helpful in the evaluation of the dynamics of pulmonary involvement in cystic fibrosis

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Table 1 *Model employed in first analysis*

The model fitted was

$\frac{1}{2}\log p/(1-p) = b\ a$	where a is age in half years
b	representing the overall mean + the effects of school 5 and social class 3
$b_1 sc1 + b\ sc2 + b\ sc4 + b\ sc5$	deviations due to other social classes
$b\ sc6 + b\ sc7 + b\ sc8$	deviations due to unemployment parental separation paternal decease
$b_9 sch1\ \ b_{10} sch2 + b\ sch3 + b\ sch4$	deviations due to other schools

girls attain menarche are estimated as 10 99 and 15 87 years (Fig 1)

If the school variables are assumed to have *no effect a significantly* worse fit (measured by χ^2 $p < 0.05$) is obtained than in the complete model *On the other hand omitting the social* variables does not make a significant difference to the χ testing goodness of fit Thus the results show no social class effect but there does appear to be some difference between schools

These findings are somewhat surprising the *differences are illustrated in* Table 2 setting out the main effects model fitted to all the data In the right hand margin appear the median values for each school if social effects are taken as zero *and* at the lower margin appear the medians for each social category ignoring differences between schools The junior school (school 5 the smallest sample) *is* perhaps by chance notably late but it does not account for the differences *between the schools for if* it is excluded differences between schools remain significant Of the *remainder* the grammar school gives the earliest mean age the catholic school the latest mean age and the comprehensive and secondary *modern* are very similar to each other Apart from the fact that these differences are not explainable by the social categories the analysis so far suggests *nothing as to their* cause

The overall values for the social categories shown at the foot of the table which *ignore the* differences between schools though not significant are of interest in that they include data relating menarcheal age to family difficulties Social classes 2 to 5 *are remarkably consistent,* and the median for girls with parents separated is very similar to these Girls with fathers unemployed appear to have the latest *menarcheal* age and where the father is dead the median is early These *suggestions obviously call for* further enquiry

Second analysis To investigate the effects of the two further factors family size and family position required a larger model and for computing *reasons it* was necessary to restrict the number of other variables Of the social variables *only social* classes 1 to 5 were considered in this analysis and the 109 children whose father was unemployed *or* dead or whose parents were separated were omitted *At* the same time social classes 1 and 2 were combined

The complete model in this analysis included variables of family order representing combinations *of* family size and position in sibship e g. first of two siblings second of three After the *complete model was fitted the null hypotheses* were tested that the school parents social class and family order alone and *in* pairs had no effect. The χ obtained are shown *in* Table 3

Again the results show no indication of a

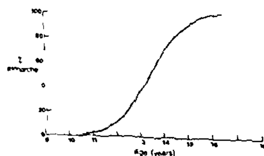


Fig 1 Proportion by age of girls who have attained menarche in South Shields.

Acta Pædiat Scand 60 158–164, 1971

AGE AT MENARCHE PHYSIQUE AND ENVIRONMENT IN INDUSTRIAL NORTH EAST ENGLAND

D F ROBERTS L M ROZNER and A V SWAN[1]

From the Laboratory of Human Genetics University of Newcastle upon Tyne and the Medical Research Council Clinical Research Centre Division of Computing and Statistics London England

In the last 20 years have appeared several studies of current age at menarche in Britain, which show a pronounced secular trend to earlier maturation and little in the way of variation from one region to another The majority of these surveys however relate to the southern part of England and there has been no investigation of a population in the industrial north east This study was undertaken to remedy this deficiency

MATERIAL AND METHODS

The study was carried out in 1967 in the urban community of South Shields County Durham and surveyed girls ranging in age from 9 to 16

For each girl was recorded the date of examination her date of birth her height weight and whether she had attained menarche Social data were also recorded—family size as indicated by the number of sibs the girl s position in the family and the social class defined by father s occupation as in the Registrar General s classification Other facts noted were whether she was a twin and where relevant supplementary social information whether the family was in economic hardship and received National Assistance whether the father was unemployed or in prison or deceased and whether the parents were divorced or separated Halfsibs stepsibs and fostersibs were all included in the measure of the family size Two girls in the care of the Local Authority and the few coloured girls were omitted giving a total sample of 1 654 subjects from these were eliminated the twins and fatherless children with mothers on National Assistance since they were so few leaving a final sample of 1 608 Five schools were included 1) a comprehensive school 2) a grammar school 3) a Catholic secondary school 4) a secondary modern school and 5) a junior school The sample thus relates to a north east English industrial urban schoolgirl population

RESULTS

1 *Menarcheal age*

The analysis consisted of the following A logit transformation $y = \frac{1}{2}\log(p/(1-p))$ was applied to the proportion p which had attained menarche at each age This was assumed to depend on a linear function of the main effects of all the variables investigated and a multiple regression technique was used to estimate these effects (e g Table 1) Groups of these effects taken in turn were then assumed zero and the significance of the additional residual heterogeneity was examined

First analysis The model was first fitted relating the logit of the proportion menstruating to variables (in the form 0 1) representing school social influences (the parents' social class paternal unemployment parental separation paternal decease) and the girls age to the nearest ½ year (Table 1) If all the variables but age are assumed to have no effect an overall median age at menarche of 13 43 years is obtained with a standard error of ±0 05 years The age limits between which 95% of

[1] *Present address* Medical Research Council Epidemiological Research Unit University of the West Indies Jamaica

Table 5 *Main effects*

Coefficient for each variable to be added to overall median and number of children on which the estimates are based

Variable		Coefficient		No of children	
Schools					
1		40		343	
2		53		614	
3		76		197	
4		0		255	
5		0		92	
Social class					
1 & 2		06		183	
3		0		737	
4		01		478	
5		13		153	
Family					
Size	Position	(*a*)	(*b*)		
1	1	04	12	146	146
2	1	0	0	199	398
	2	16		199	
3	1	4	26	136	374
	2	43		143	
	3	34		95	
4	1	[illegible]	47	53	[illegible]
	2 & 3	37		131	
	4	43		36	
5	1	44	45	59	363
	mid	47		281	
	last	55		23	

To estimate the median menarcheal age for any group of girls in this sample three coefficients must be employed one from each of the sections school social class and family (column (*a*) if sibship position is required column (*b*) if it is not)

2 *Physique*

The inter relations of age at menarche physique and the environment in which girls develop are complex and from data that are not longitudinal and therefore cannot include

Table 6 *Second and third analyses*

Median age at menarche when family size is the sole variable analysed

Family size	Median
1	13 04
[illegible]	13 14
3	13 45
4	13 68
5 or more	13 66

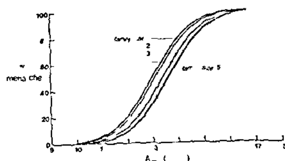


Fig 2 Effects of family size on menarcheal age

growth velocities comprehensive analysis is not possible As a more limited objective the South Shields data were analysed to discover which of the variables previously considered exerted any *detectable influence on height weight* and the ponderal index *(height / $\sqrt[3]{\text{weight}}$) calculated* therefrom This was done by obtaining the multi linear regressions of each of the three physique variables in turn on variables in the form 0 1 representing the schools social class family position and menarcheal state The age of the girls introduced a complication for there is during puberty a non linear relationship between the physique variables and age To overcome this the data were divided into yearly age groups and the regressions performed separately within each of these groups This procedure allows age to be considered as held constant albeit approximately and omitted from the analysis while the effects of the other variables are investigated

In the age range 10 to 11 years there are *46 children none of whom has reached men*arche The only physique variable that shows a significant regression in this age range is the *ponderal index* ($p < 0.05$) for there is a tendency for the index to increase with the family *order variables* The analysis was repeated with *family size variables replacing the family order* (*combined* size and position) variables again the regression of ponderal index on these is significant (p 0 05) As in the menarche analysis it seems to be the size of the family rather than the position of the child in that family which influences ponderal index in this age

Table 2 *Median age at menarche*

First analysis Main effects model fitted to all data (ages in years)

	Social class					Father unemployed	Parents separated	Father deceased	Overall
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	
School 1	12 47	13 46	13 48	13 50	13 37	13 66	13 48	12 80	13 46
2	12 29	13 28	13 30	13 32	13 19	13 48	13 30	12 62	13 76
3	12 74	13 73	13 75	13 77	13 64	13 93	13 75	13 07	13 74
4	12 43	12 42	13 44	13 46	13 33	13 62	13 44	12 76	13 43
5	13 05	14 05	14 06	14 08	13 95	14 25	14 06	13 38	14 04
Overall	12 30	13 37	13 43	13 49	13 39	13 67	13 40	12 74	

social class effect Neither in this analysis do they show any indication of a school effect either alone or combined with social class On the other hand they do show a significant family order effect ($p < 0\ 025$)

Third analysis A reduced model in which the family order variables were replaced by variables representing family size alone made it possible to test the significance of position in sibship and family size separately and the results are shown as subdivisions of the family order χ^2 in Table 3 The family order effect is almost entirely due to the family size effect which is highly significant ($p < 0\ 005$) while the position in sibship does not appear to matter These results suggest quite strongly that the significant school effect obtained in the first analysis was the result of systematic differences in family size from school to school This is illustrated by the family sizes for each school given in Table 4 the differences between the schools are pronounced

The coefficients of the various factors in the second and third analyses and the numbers of children on which they are based are set out in Table 5 These coefficients are to be added to the overall median to obtain that for any particular category of child e g girls at school 2 of social class 4 who are the second in a family of three have a median menarcheal age of $13\ 43 - 0\ 53 - 0\ 01 + 0\ 43 = 13\ 32$ years Since of these the only factor found to be of significant effect was family size the medians when family size alone is analysed are set out in Table 6 and the distributions in Fig 2

In summary then the results show no independent effect of social class of school or of family position In the South Shields data the two main factors of those analysed that influence whether a girl has attained menarche or not are her age and the size of the family from which she comes

Table 3 *Second and third analyses*

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	χ^2	d f	p
Complete model	611 78	761	
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No family position or size effect	22 27	11	<0 025
No family position effect	4 00	7	
No family size effect	18 27	4	<0 005
No social class effect	0 70	3	
No school effect	4 03	4	
No school or social class effect	4 65	7	

Table 4 *Numbers of children with a given family size within schools with the mean family sizes*

School	Family size 1	2	3	4	5+	Mean size
1	37	92	86	59	69	3 3±0 10
2	86	228	154	70	76	2 8±0 06
3	3	21	41	40	92	4 6±0 15
4	16	47	57	33	102	4 4±0 16
5	4	11	35	18	24	3 8±0 16

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3		0		737	
4		– 01		428	
5		– 13		153	
Family					
Size	Position	(*a*)	(*b*)		
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2	1	0	0	199	398
	2	– 16		199	
3	1	74	– 26	136	374
	2	+ 43		143	
	3	– 34		95	
4	1	2	47	53	270
	2 & 3	– 32		131	
	4	– 43		36	
5	1	– 44	45	59	363
	mid	47		281	
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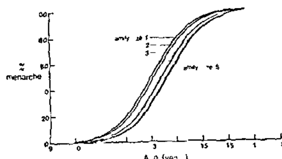


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First analysis Main effects model fitted to all data (ages in years)

	Social class					Father unemployed	Parents separated	Father deceased	Overall
	1	2	3	4	5	6	7	8	
School 1	12.47	13.46	13.48	13.50	13.37	13.66	13.48	12.80	13.46
2	12.29	13.28	13.30	13.32	13.19	13.48	13.30	12.62	13.26
3	12.74	13.73	13.75	13.77	13.64	13.93	13.75	13.07	13.74
4	12.43	12.42	13.44	13.46	13.33	13.62	13.44	12.76	13.43
5	13.05	14.05	14.06	14.08	13.95	14.25	14.06	13.38	14.04
Overall	12.30	13.37	13.43	13.49	13.39	13.67	13.40	12.74	

social class effect Neither in this analysis do they show any indication of a school effect, either alone or combined with social class On the other hand they do show a significant family order effect ($p < 0.025$)

Third analysis A reduced model in which the family order variables were replaced by variables representing family size alone made it possible to test the significance of position in sibship and family size separately and the results are shown as subdivisions of the family order χ in Table 3 The family order effect is almost entirely due to the family size effect which is highly significant ($p < 0.005$) while the position in sibship does not appear to matter These results suggest quite strongly that the significant school effect obtained in the first analysis was the result of systematic differences in family size from school to school This is illustrated by the family sizes for each school given in Table 4, the differences between the schools are pronounced

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No family size effect		18.27		4	<0.005
No social class effect	0.70		3		
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4	16	47	57	33	102	4.4±0.16
5	4	11	35	18	24	3.8±0.16

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	2 & 3	– 37		131	
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5	1	44	45	59	363
	mid	47		81	
	last	– 55		23	

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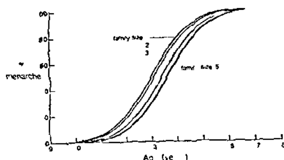


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3	12 74	13 73	13 75	13 77	13 64	13 93	13 75	13 07	13 74
4	12 43	12 42	13 44	13 46	13 33	13 62	13 44	12 76	13 43
5	13 05	14 05	14 06	14 08	13 95	14 25	14 06	13 38	14 04
Overall	12 30	13 37	13 43	13 49	13 39	13 67	13 40	12 74	

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5	4	11	35	18	24	3 8 ± 0 16

The results over the whole age range in these children indicate that of the factors investigated only menarcheal status has a consistent effect on physique Table 9 gives the estimated effects of menarche on height and weight for each age group and the numbers of children The four girls in the youngest age group who are ahead of their age mates in menarche are also very much ahead of them in stature while the seven girls in the 15 to 16 age group who are lagging in maturation are also considerably shorter suggesting that it is at the extremes of maturation that its relationship with physique may be most pronounced

DISCUSSION

Menarche

The variables examined in this survey were chosen as a result of some rather unexpected findings of an investigation amongst Swansea college girls (4) where the method of survey was by recall on a series of cross sectional samples Besides a secular trend in menarcheal age significant effects were detected of family size and position in family but there was no effect of social class It was obviously necessary to substantiate these findings on a sample at which the criticism that the data were open to recollection error could not be levelled and the method employed here collecting data on whether menarche had or had not been attained with subsequent logit analysis was the obvious choice

The South Shields results do in fact provide reasonable confirmation They endorse the absence of a social class effect on menarcheal age and are in this compatible with other recent studies in Britain (2 3) The family size effect again confirms the Swansea findings not only in its occurrence but also in its magnitude in the present sample (family sizes 1 to 4 inclusive) averaging 0 18 years per additional sib by comparison with 0 154 ± 038 years per additional sib in the Swansea results The South Shields findings however differ from the Swansea results in the absence of a significant family position effect yet inspection of the family position coefficients in Table 5 suggests that in the largest families (of size 4 and size 5 and above) the youngest sib may indeed be accelerated by comparison with the oldest and middle sibs though this is not sufficiently marked to be distinguished from chance fluctuations in this sample Further investigation of larger families is clearly required

Taken overall these findings like those of the Swansea investigation suggest that environment (and in particular standards of nutrition and general care) is still an important determinant of age at menarche but that today in Britain it operates through family size and no longer through accepted socio-economic categories The Registrar General s categories are today it seems a less effective measure than formerly of differences in housing standards expenditure on food and other variables of potential biological relevance To these instead family size appears to be a much more sensitive indicator poverty that is effective in that it influences biological development should today perhaps be sought among those of large families instead of those of the lower classes However it is possible that some other factor not considered in this study e g some genetic influence may be affecting families of different sizes by different amounts

The overall median age (13 43 years) is in fact later by one third of a year than that in the last reported status quo sample from Britain (6) This delay may perhaps indicate an interruption or termination of the secular trend to earlier menarche On the other hand it may be merely a reflection of the characteristics of this sample drawn from an urban industrial area where there is still much economic hardship The latter interpretation is compatible with the environmental effect on maturation postulated above Or perhaps the delay is due to a combination of both

Physique

The number of analyses undertaken in the physique investigation colours their interpretation

Table 7 *Mean ponderal indices against family size for children in the 10–11 year age group*

Family size	Mean P I
1	12 38
2	12 77
3	12 77
4	13 27
5+	12 59

Table 8 *Mean age by schools within the 11–12 year age group*

School	No of girls	Mean age in years
1	30	11 83
2	17	11 91
3	23	11 77
4	12	11 88
5	37	11 30

group The mean ponderal indices are given in Table 7

In the age range 11 to 12 years (119 children) the regressions of both height and weight are significant ($p<0\ 01$) due partly to differences between schools (height $p<0\ 01$, weight $p<0\ 05$) and social classes (height and weight $p<0\ 05$), but mainly menarcheal status (height and weight $p<0\ 001$) However, this group includes 37 children from the junior school (5) whose average age and consequently their average height and weight within the one year age group is considerably less than those of the children in the other four schools (Table 8) When these children are omitted the significance of the differences between schools disappears and so does the effect of social class on height This leaves only the regressions of height and weight on menarche and of weight on social class as significant This last appears to be due to the fact that in this age grou the few children in social classes 1 and 2 a particularly heavy in their combined averag about 25 lbs heavier than children of equivale menarcheal state in the other social classe The finding in this age group that menarche status (whether the child had reached menarcl or not) has a significant effect on height ai weight but not on ponderal index is surprisii in view of the consistent decrease in the indi associated with menarche found in the 12–: age groups

In the age ranges 12 to 13, 13 to 14 and i to 15 years with 304 366 and 351 childr respectively all the regressions are significa ($p<0\ 01$) but solely on account of the effe of menarcheal state except for an isolated i stance in the range 14 to 15 years The exce tion in this range is the regression of height c school which is also significant ($p<0\ 01$) a parently on account of the children in the se ondary modern school (4) being on average oi inch shorter than children in the other thr schools, even after allowance for social cla and menarcheal state

In the age ranges 15 to 16 and 16 years i more with 216 and 90 children respectivel the regressions of ponderal index are no long significant and in the latter range neither is tl regression of height Those regressions whic are significant in these age ranges are aga solely due to the regression on menarche state In these groups only seven and one gir respectively have not reached menarche so di ferences between those who have and those wh have not would have to be very large to reach significance

Table 9 *Estimated effects of menarche on height and weight*

			Increase due to menarche of	
Age group	No of children	No menstruating	Height (ins)	Weight (lbs)
11–12	82	4	6 2±1 3	33 5±8 6
12–13	304	76	2 1±0 4	18 2±2 4
13–14	366	178	2 0±0 3	21 6±1 9
14–15	351	285	1 6±0 3	18 9±2 3
15–16	216	209	3 2±1 0	21 5±7 5

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TEST MEAL IN THE DIAGNOSIS OF MALABSORPTION IN INFANCY

Tolerance Tests Using Simultaneous Oral Administration of Glucose D Xylose Cream and Vitamin A

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In the clinical routine work up of patients with symptoms suggesting a malabsorption syndrome a series of tolerance tests is usually performed in order to obtain a pathogenetic diagnosis Two of the major diagnostic tests are the D xylose (9) and vitamin A (1) tests Usually glucose tolerance test is also performed (11) Cream is sometimes preferred to vitamin A as a test of fat absorption (3)

The purpose of this communication is to report studies on the diagnostic value of a test meal containing glucose D xylose cream and vitamin A in infants with various disorders causing malabsorption syndrome Normal infants and patients with cystic fibrosis extra hepatic biliary atresia intrahepatic cholestasis (i e neonatal hepatitis) and gluten induced enteropathy have been examined The amount of the individual constituents of the meal was similar to those usually given in isolated tolerance tests Following the combined test meal the blood concentrations of glucose D xylose triglycerides and vitamin A have been determined

MATERIAL

Normal infants

Sixteen infants 9 boys and 7 girls with normal growth pattern were studied There was no evidence of infection anemia or gastrointestinal disturbances

This study has been supported by grants from the Swedish Medical Research Council (60) from Stockholms Lans Landsting and from Karolinska Institutets reservationsanslag

Infants with malabsorption syndromes

Three infants with *cystic fibrosis* 3 with *extrahepatic biliary atresia* and 4 with *intrahepatic cholestasis* (neonatal hepatitis) were studied

Four infants with gluten induced enteropathy (coeliac disease) were studied before a gluten free diet was instituted All of them responded to the gluten free diet and developed clinical symptoms upon provocation with gluten

Clinical data are given in Table 1

METHODS

Cholic acid 24- C was administered intramuscularly to the patients with cholestasis The urinary and faecal isotope excretion was determined as described earlier (12)

Analytical methods are given in Table 2

TEST-MEAL

The infants received a diet regular for their ages during the 3 days preceding the test meal Infants younger than 2 months of age fasted 6 hours older infants 9 hours A feeding tube was then put into the stomach through the nose and vitamin A palmitate was given through the feeding tube immediately followed by a solution containing glucose D-xylose and cream The amount of the four constituents administered was as follows vitamin A palmitate (Ido-A® Ferrosan 100 000 IU per ml) 7 500 IU per kg body weight glucose 2 g per kg body weight (with a minimum dose of 20 g) D-xylose 0 5 g per kg body weight standardized cream (12% fat) 8 ml per kg body weight

The infants were not allowed to eat or drink 4 hours after the test meal Water was then allowed and after another hour the infants received a regular meal

Capillary blood samples were taken at fasting 30

Altogether 21 regression analyses were performed (the three dependent physique variables in each of the seven age groups) and significant differences appeared between schools between social classes and between family positions on three occasions, once each Since on average a significant result using a 5% level will be obtained by chance one in twenty times these results should be regarded with some circumspection Quite different however is the effect on physique of menarcheal status Ignoring the age groups 10 to 11 in which there are no girls who have achieved menarche, and 16+ in whom there is only one who has not in the remaining five groups the effect of menarcheal status on stature and weight is significant in each while its effects on ponderal index are significant in the three middle age groups 12 to 13, and 14 to 15, but not in the 11 to 12 or 15 to 16 groups For the discrepancy in these two it seems that the height change is so pronounced relative to that in weight that the index remains relatively constant for it is in these two groups that the greatest differences in stature with menarcheal status occur (Table 9) Overall however the effects of menarcheal status on physique are consistent and pronounced Girls who have attained menarche are consistently heavier and taller in each age group than those who have not the difference in height being particularly pronounced in the case of girls most extreme in maturation age The magnitude of the overall effect on weight is perhaps not unexpected in view of the growth in the many different tissues that is occurring at puberty

The absence of effect of any of the other variables is surprising From the other surveys of physique in relation to environmental variables one might have expected some effect of social class (e g 1 7), or of family size (5) and these do not occur Their absence may be partly due to the fact that even in a total sample of this size by the time the data are broken down by age groups and menarcheal status the sub samples are small and numbers insufficient to show any significant effect

SUMMARY AND CONCLUSIONS

A survey of age at menarche how it is affect by family environment and how it affe physique, was carried out in 1967 on a la sample of schoolgirls in the urban industr town of South Shields County Durham A at menarche shows no independent effect social class of school or of position in sibsh but is strongly influenced by the size of fam in which a girl grows up Menarche is associat with consistent and pronounced increments height and weight in the presence of which consistent effects of variables of the family e vironment on physique can be detected

ACKNOWLEDGMENTS

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Key words Age at menarche industrial area family environment

Table 2 *Scheme of sampling and analytical methods after test meal*

Analysis	Method (reference)	Total amount of blood required (ml)	Time of analysis after administration of the test meal (minutes)									
			30	60	90	1 0	180	240	300	360	480	600
Glucose	(5)	0 1	×	×	×	×	×					
D xylose	(9)	0 1	×	×	×	×	×	×				
Triglycerides	(10)	0 4				×	×	×	×			
Vitamin A	(7)	0 4						×		×		×

= analysis

grouped according to the ages of the infants and are presented in Fig 1 Because of the small number of cases and the great individual variability in the youngest normal infants a statistical analysis of the results was not possible Th individual variations were most pronounced in the youngest infants

Glucose The blood concentration rose to mu h higher values in the youngest infants than in those being 13 to 20 months of age which is most likely explained by the fact that they rec ived a greater amount of glucose in relation to their body weights In 15 of the 16 infants studied the peak concentration was observed within 60 min after the administration of the test meal In 12 of the 16 infants the blood concentration dropped to a level below 100 mg per 100 ml within 120 min

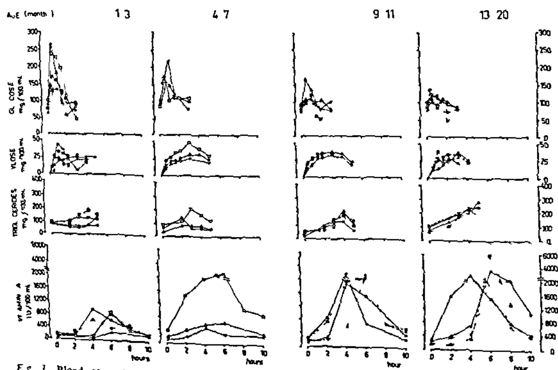


Fig 1 Blood concentrations of glucose D xylose triglycerides and *vitamin A* after test meal given to normal infants of varying age From the left to the right ○—○ ♂ 1 month of age △—△ ♀ 1 ½ months of age □—□ ♂ 2 months of age ■ ■ ♂ 3 months of age ● ● ♀ 3 months of age ○—○ ♂ 4 months of age ▲—▲ ♂ 4 months of age □—□ ♂ 7 months of age ○—○ ♀ 9 months of age △—△ ♀ 9 months of age ■ ■ ♂ 9 ½ months of age ●--● ♂ 10 months of age ○—○ ♀ 13 months of age △—△ ♀ 13 months of age ■ ■ ♂ 16 months of age ●--● ♀ 20 months of age

Table 1 *Clinical data in infants with malabsorption*

Diagnosis	Case no	Sex	Age (mo)	Weight (kg)	Length (cm)	Remarks
Cystic fibrosis	1	♀	1¼	2 4	48	Meconium ileus Chymotrypsin = 110–300 μg/g faeces Clorides/sweat[c] = 128 mM
	2	♂	6½	6 5	64	Chymotrypsin = 26 μg/g faeces Clorides/sweat[c] = 101 mM
	3	♀	19	9 6	80	Chymotrypsin = 20 μg/g faeces Clorides/sweat[c] = 112 mM
Extrahepatic biliary atresia	4	♀	3	5 0	62	Atresia of D hepatici and D choledochus[d]
	5	♂	4½	4 8	62	Atresia of D hepatici and D choledochus[d]
	6	o	10	5 3	62	Atresia of D hepatici and D choledochus[d]
Intrahepatic cholestasis (Neonatal hepatitis)	7	o	5	4 8	60 5	Persisting jaundice hepatosplenomegaly Congenital heart disease Operative cholangiography showed normal bile ducts
	8	♀	3	3 6	54	Jaundice hepatomegaly Susp congenital heart disease After 4 months of age regression of jaundice
	9	♂	13½	8 2	71	Persisting jaundice hepatosplenomegaly Operative cholangiography showed normal bile ducts
	10[a]	♀	5½	4 7	59	Jaundice Hepatosplenomegaly Congenital heart disease Operative cholangiography showed normal bile ducts
Gluten induced enteropathy Before gluten free diet	11	♀	6	6 1	67	Poor weight gain Protruding abdomen Voluminous stools Follow up showed clinical improvement after gluten free diet Positive gluten provokation
	12	♂	9	8 0	69 5	Poor weight gain Protruding abdom n Voluminous stools Follow up showed clinical improvement after gluten free diet Positive gluten provokation
	13	♂	10¼	7 3	70 5	Poor weight gain Protruding abdomen Voluminous stools Follow up showed clinical improvement after gluten free diet Positive gluten provokation
	14[b]	♂	7	6 0	66	Poor weight gain Protruding abdomen Voluminous stools Follow up showed clinical improvement after gluten free diet Positive gluten provokation

[a] Re-examined at 20 months of age when clinically healthy
[b] Re-examined at 13 months of age when clinically healthy Between the examinations the infant was on a gluten free diet
[c] Pilocarpin iontophoresis
[d] Verified at laparatomy and autopsy

60 90 120 180 240 300 360 480 and 600 min after the test meal The amount of blood required and the time for analysis of each parameter is given in Table 2

RESULTS

Normal infants

The results of each of the parameters determined in the 16 normal infants have been

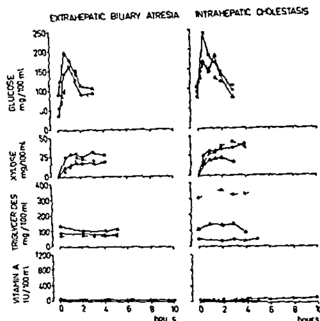


Fig. 4. Blood concentrations of glucose, D-xylose, triglycerides and vitamin A after test meal given to infants with malabsorption syndromes. Extrahepatic biliary atresia: ○—○ case 4, △—△ case 5, ●---● case 6. Intrahepatic cholestasis: ○—○ case 7, △—△ case 8, ●---● case 9. For data on the patients see Table 1.

infants. In two patients the blood concentration of *D-xylose* increased above 24 mg per 100 ml; in the third the maximum concentration was 20 mg per 100 ml. No increase in the blood concentration of *triglycerides* or *vitamin A* was seen in these 3 infants (Fig. 4).

Intrahepatic cholestasis. As can be seen from Fig. 4 the maximum increase in blood *glucose* concentration was comparable to that found in normal infants. The maximum blood concentration of *D-xylose* was above 24 mg per 100 ml. The blood concentration of *triglycerides* remained unchanged in two infants and increased in one infant 25% above the fasting level. One of the infants had a high fasting level. The blood concentration of *vitamin A* did not rise significantly in any of the three infants.

Gluten induced enteropathy. In only one of the three patients was the increase in blood *glucose* concentration 30 min after the test meal comparable to that seen in normal infants (Fig. 3). In the other two infants the increase was only 6 and 14 mg per 100 ml above fasting level. The maximum blood *D-xylose* concentrations were 7, 15 and 19 mg per 100 ml. [illegible] ration of *triglycerides* did not increase in two patients; in the third the maximum increment was 43% above fasting level. There was a slight increase of the blood concentration of *vitamin A* 6 hours after the test meal in only one patient.

Consecutive studies in individual patients

The test meal was used to monitor intestinal absorption during the course of a disease and

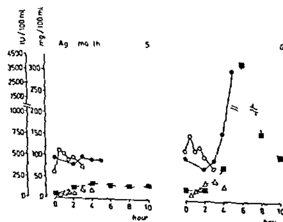


Fig. 5. Blood concentrations of glucose, D-xylose, triglycerides and vitamin A after test meal in an infant with intrahepatic cholestasis (case 10, Table 1). Symbols: ○—○ glucose, △—△ D-xylose, ●—● triglycerides, ■---■ vitamin A.

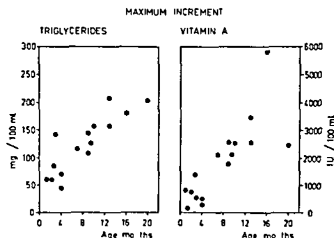


Fig 2 Maximum increases above fasting levels of triglycerides and vitamin A after test meal given to normal infants

D xylose The highest blood concentration was usually reached within 2 to 3 hours after the test meal In all infants the maximum blood concentration was above 24 mg per 100 ml In two of the youngest infants $1^{1}/$ and 3 months of age there were two peaks

Triglycerides The blood concentration reached a maximum within $4\,^{1}/_{2}$ hours in 13 of the 16 infants The maximum increment of triglycerides increased with increasing age (Fig 2) The percentage peak increment exceeded 63% in 15 of the infants studied

Vitamin A Maximum blood concentration was reached within 4 to 6 hours after the test meal in all infants The maximum increment of vitamin A increased with age (Fig 2) Below 4 months of age the maximum blood concentration never exceeded 1 600 IU per 100 ml on the other hand in all infants above this age the peak blood concentration exceeded this level

Infants with malabsorption syndromes

Cystic fibrosis In the three infants studied the blood concentration of *glucose* and *D xylose* rose to the same level as found in normal infants of comparable ages In two of the infants the blood concentration of *triglycerides* decreased initially After 3 hours it returned to the fasting level in the first infant and to a value which was 13% above that level in the second infant In the third patient there was an increase of 20 mg per 100 ml corresponding to an increment of 13% No increase of the blood concentration of *vitamin A* was observed (Fig 3)

Extrahepatic biliary atresia The magnitude of the increases and the pattern of changes in blood concentrations of *glucose* in these 3 infants were comparable to those seen in normal

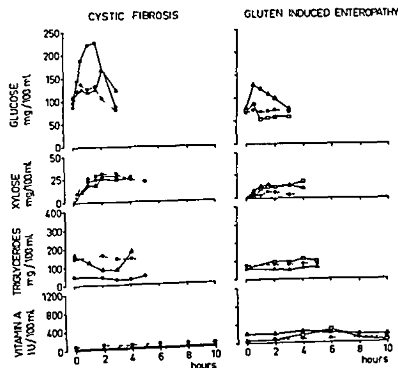


Fig 3 Blood concentrations of glucose D xylose triglycerides and vitamin A after test meal given to infants with malabsorption syndromes Cystic fibrosis ○—○ case 1 △—△ case 2 ●--● case 3 Gluten induced enteropathy ●--● case 11 △—△ case 12 □—□ case 13 For data on the patients see Table 1

bined tolerance test for D xylose and cream similar results have been obtained (4 b) The increases in the blood concentration of triglycerides were small in the youngest infants and a comparison to pathological states was not possible unless the percentage increment was calculated In 15 of 16 infants this was found to be greater than 63 % a value never reached in any of the infants with abnormal conditions studied Disturbances in fat absorption in this age group however were readily detectable in the pattern of vitamin A responses (cf 4 a 13)

It is well known that infants with cystic fibrosis have an impaired absorption of triglycerides and vitamin A but normal absorption of D xylose The expected results were thus obtained in our 3 patients studied with the test meal

In the infants with extrahepatic biliary atresia and intrahepatic cholestasis a disturbance of fat absorption is expected due to an impairment of bile acid excretion to the intestine (12) This is well illustrated by the changes in blood concentration of triglycerides and vitamin A after the test meal (Figs 4 and 5) In some of the patients with cholestasis the blood concentration of D xylose was low compared to normal infants of the same age Since D xylose tolerance test is thought to reveal mucosal damage which is not expected to occur in these conditions this finding is difficult to explain However the fact that abnormal D xylose tolerance tests have been observed in adults with cirrhosis of the liver (15) may indicate that the absorption of D xylose is less efficient in liver disease Bile acid excretion was not studied in the adults with cirrhosis of the liver From our results obtained in the infant with intrahepatic cholestasis studied twice it is evident that the D-xylose triglycerides and vitamin A responses to the test meal became normal when bile acid excretion to the intestines had normalized spontaneously

In gluten induced enteropathy with atrophy of the intestinal mucosa the absorption of fat as well as carbohydrates is known to be impaired The present results using the combined test meal are in agreement with those previously reported using single tolerance tests The blood concentration of D-xylose never rose to values observed in normal infants In the patient studied twice the absorption of fat and carbohydrates improved markedly after treatment with a gluten free diet

There may be several advantages of the combined test meal compared to single tolerance tests Since the test meal is comparable with a regular meal for the infant a prolonged partial fasting is avoided It is considerably easier to follow the absorption of fat and carbohydrates during the course of a disease and during a therapeutic regimen when a series of single tolerance tests is replaced by one test e g a test meal

SUMMARY

A test meal containing 2 g of glucose per kg body weight (minimum of 20 g) 0 5 g of D xylose per kg 8 ml of standardized cream (12 % fat) per kg and 7 500 IU of vitamin A palmitate per kg was administered through a nasogastric tube During the next 10 hours capillary blood samples were obtained and analysed for glucose D xylose triglycerides and vitamin A using microanalytical methods

The results in 16 normal infants aged 1 to 20 months are reported The results for all parameters determined except D xylose were comparable to those previously reported using single tolerance tests The variability related to age was pronounced for glucose triglycerides and vitamin A

In patients with malabsorption syndromes (cystic fibrosis extrahepatic biliary atresia intrahepatic cholestasis and gluten induced enteropathy) the results were also comparable with those earlier observed using single tolerance tests

A combined test meal has certain advantages over the examinations now used in infants with symptoms suggesting a malabsorption syndrome a series of tolerance tests is avoided and it is therefore easier to follow the absorption

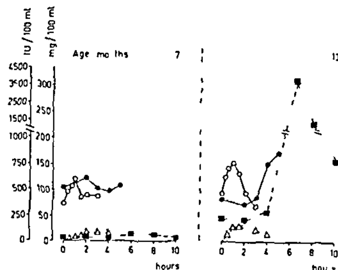


Fig 6 Blood concentrations of glucose D xylose triglycerides and vitamin A after test meal in an infant with gluten induced enteropathy (case 14 Table 1) Symbols O—O glucose Δ Δ D xylose ●—● triglycerides ■---■ vitamin A

to evaluate the effect of a therapeutic regimen Two illustrative cases are shown in Figs 5 and 6

In one infant with intrahepatic cholestasis (case 10 Table 1) the test meal was given at 5 months of age when almost all administered cholic acid 24 ^{14}C was recovered in the urine, and again at 20 months of age when almost none was excreted in the urine (Fig 5) At 5 months of age the maximum increase in the blood D xylose concentration was below 24 mg per 100 ml, the increase in the blood concentration of triglycerides was only 19% and only a very slight (90 IU) increase in the blood concentration of vitamin A was observed At 20 months of age the blood concentration of D xylose triglycerides and vitamin A rose to levels comparable with those seen in a normal infant of the same age

One infant with gluten induced enteropathy (case 14, Table 1) (an intestinal biopsy specimen at 7 months of age showed typical mucosal atrophy) had only a slight increase in the blood concentration of D xylose triglycerides and vitamin A (Fig 6) The maximum blood concentration of D xylose was 11 mg per 100 ml 3 hours after the test meal After 6 months on a gluten free diet, marked increase in the blood concentration of D xylose, triglycerides, and vitamin A above fasting levels was found The maximum blood concentration of D xylose (36 mg per 100 ml) was seen 2 hours after the test meal

DISCUSSION

In normal infants the changes in the blood concentration of D xylose and especially of glucose, triglycerides and vitamin A following the test meal appeared to vary with age

The high blood glucose concentrations in the youngest infants were probably a reflection of the relatively greater dose of glucose given per kg body weight Influence of the other constituents in the test meal on the changes in the blood glucose concentration seems to b negligible, since their curves are otherwise comparable to those observed during simple glucose tolerance tests at these ages (2 11)

The blood concentration of the D xylose seems, to some extent to be influenced by the other components of the test meal In the normal infants the blood D xylose concentration was low compared to those seen in ordinary D xylose tolerance tests (9), in addition the maximum blood concentration using the combined test meal occurred relatively late It may be speculated upon if these findings can be explained at least partly by delayed gastric emptying which has been shown to occur in the presence of high concentrations of glucose (6) This may be one reason for the great individual variation in the youngest infants A competitive inhibition of the intestinal active transport of D xylose by glucose may also be considered since such a phenomenon has been shown to occur *in vitro* (14)

The gradual increase of vitamin A response with the age of the infant as found in the combined test meal is in accordance with results obtained in single vitamin A tolerance tests (8 16)

The variations in blood concentrations of triglycerides with the age of the infant were also considerable as shown in Fig 2 Using a com

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THE SIGNIFICANCE OF COPROANTIBODIES TO COW S MILK PROTEINS

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Sensitivity to cow s milk protein appears to be an allergic phenomenon It is diagnosed mainly on clinical grounds The development of antibodies to cow s milk in the serum is a physiological process and does not in itself signify sensitivity to cow s milk (8 20) If the titers of antibodies to cow s milk are very high however they usually do indicate some pathology They may be found in milk sensitivity (9 16 24) in celiac disease (1 15 23) in mongolism (19) in dysautonomia (6) and in other chronic diseases (21) Since serological tests have not proved to be diagnostic a search for milk antibodies in feces (coproantibodies) in children with gastrointestinal milk allergy seemed logical (2 17) We will report here on the value of coproantibodies in distinguishing gastrointestinal milk allergy from other conditions in which there are high levels of milk antibodies in serum The immunoglobulin nature of the serum and coproantibodies to the proteins of cow s milk was also investigated

MATERIAL AND METHODS

Four groups of children were investigated Group 1 consisted of healthy infants under the age of 1 year seen during a prospective study of immunological responses to milk protein Group 2 was comprised of children with a variety of acute or chronic conditions admitted to the Children s Department of Shaare Zedek General Hospital Group 3 consisted of children with mongolism or dysautonomia Group 4 consisted of children with gastrointestinal milk sensitivity or celiac disease

The children with milk sensitivity suffered from diarrhea and vomiting which ceased when cow s milk was replaced by a soybean milk preparation The diagnosis was confirmed by three oral challenges with cow s milk and subsequent oral challenges with isolated milk proteins Most of the patients have been described in detail elsewhere (9)

Five were sensitive to beta lactoglobulin one to bovine serum albumin and one to casein Celiac disease was diagnosed by fat balance intestinal biopsy and response to gluten free diet

Stools were centrifuged twice at 37 000 *g* for 45 min Capillary blood was obtained *in group 1* and venous blood in the other groups

The following antigens were used —pasteurized whole milk centrifuged twice at 1 000 *g* for 10 *min* and once at 37 000 *g for 30 min in* the cold to remove fat and precipitable particles Beta lactoglobulin crystallized and alpha lactalbumin purified were purchased *from Pentex Co* Kan Kakee Ill bovine serum albumin crystallized and bovine gamma globulin from Armour Pharmaceutical Co Chicago Ill Casein 10 times crystallized was kindly supplied by Dr S Saperstein Pharmaceutical Division the Borden Co New York N Y

For passive hemagglutination the technique of Boyden (3) was employed with some modifications (13) Radio-immunoelectrophoresis was done by the method of Yagi et al (25) The antigens were iodinated with ^{125}I by the chloramine T method To every slide 0 1 ml of iodinated antigen was added The concentration of the iodinated milk bovine gamma globulin and beta lactoglobulin was 10-20 μg/ml and that of alpha lactalbumin and bovine serum albumin 5-10 μg/ml Each gamma of the iodinated antigen contained 5-10 μCi of I^{125} Weak antigen binding was shown by the arc of alpha globulin in some tested sera A similar reaction was noted by Yagi

This study was supported by research grant FG IS-37 from the US Department of Agriculture

of fat and carbohydrates during the course of a disease and during a therapeutic regimen

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Key words Malabsorption syndromes infants D xylose glucose tolerance test vitamin A tolerance test plasma triglycerides

Table 3 *Incidence of precipitating serum and coproantibodies to milk proteins (all groups)*

	Serum						Feces					
	Whole milk	ALA	BLG	BSA	BGG	Casein	Whole milk	ALA	BLG	BSA	BGG	Casein
Healthy controls	4/104	6/104	3/104	0/104	4/104	0/104	0/21	2/21	0/21	0/21	0/21	0/21
Other diseases[a]	2/14	3/14	1/14	2/14	1/14	0/14	0/34	2/34	0/34	0/34	0/34	0/34
Mongolism and dysautonomia	5/6	5/6	2/6	0/6	2/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
Milk allergy												
Prolonged exposure	5/7	5/7	3/7	2/7	4/7	0/7	3/7	2/7	2/7	1/7	1/7	0/7
Short exposure	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Celiac disease	3/4	2/4	2/4	1/4	1/4	2/4	1/4	1/4	1/4	0/4	0/4	0/4

[a] See first and last foot notes in Table 1
Numbers indicate positive reactions / total number of children examined

tions did not vary significantly from that in healthy controls Over the age of 1 year coproantibodies were found in only one out of ten control cases tested

The children with mongolism and with dysautonomia were analysed together as their immunological reactions were similar and we considered that aspiration of milk is a common facto in both The incidence of coproantibodie was small while the incidence and the levels of antibodies in erum were abnormally high

Table 2 describes hemagglutination reactions in milk allergy and celiac disease When exposure to milk has been sustained for over 3 weeks high levels of antibodies are found in serum and feces in all cases When exposure to milk did not exceed a few feeds the antibody response did not differ markedly from that of normal controls In celiac di ease mean antibody titers in serum and feces tended to be rather higher than in controls We could find no evidence in any group that serum and coproantibodies were consistently directed against the ame antigens Furthermore antibody response gave no indication which of the milk proteins was responsible for symptoms of milk allergy

The general pattern of the hemagglutination reactions is also reflected by our studies of serum and coproantibodies by precipitation in gel (Table 3) The very high incidence of serum antibodies to milk in mongolism and dysautonomia is contrasted by the absence of coproantibodies in these conditions On the other hand in gastrointestinal milk allergy prolonged exposure to milk resulted in a raised incidence of precipitating antibodies in feces and serum This correlation between serum and coproantibodies held true for celiac disease as well

The immunoglobulin nature of the antibodies to milk proteins were studied by radio-immunoelectrophoresis (Fig 1) The responses in normal children and children with various acute and chronic conditions were similar and they are therefore considered as one group (Table 4) Serum antibodies were frequent and were of the IgG class Coproantibodies to milk were found in 1 case only and were of the IgA class (Fig 2)

A similar pattern prevailed also in mongolism and dysautonomia and was quite different from that obtained in milk allergy and celiac disease Although in the last two conditions erum antibodies were also mainly of the IgG class it was only here that serum milk antibodies belonging to the IgA class could be found Coproantibodies to milk were the rule among milk sensitive children after prolonged exposure There were derived mainly from IgA and to a lesser extent from IgG

The similarity of immunoglobulins in serum and feces was proven by immunodiffusion ana

Table 1 *Hemagglutinating serum and coproantibodies to milk protein groups 1 2 and 3*

Group	Serum						Feces					
	Whole milk	ALA[a]	BLG	BSA	BGG	Casein	Whole milk	ALA	BLG	BSA	BGG	Casein
Healthy controls (1-12 months old)	1 8[b] (9/10)	3 4 (10/10)	1 9 (8/10)	2 9 (9/10)	1 1 (9/10)	2 3 (10/10)	0 9 (6/15)	0 7 (4/15)	0 1 (2/15)	0 5 (4/15)	1 3 (11/15)	1 (6 15)
Other diseases [c]	1 9 (15/20)	2 3 (15/20)	2 3 (14/18)	2 6 (17/20)	1 6 (13/19)	1 8 (12/20)	0 6 (3/22)	0 9 (8/22)	0 7 (6/22)	0 4 (4/22)	1 6 (9/22)	1 1 (11/22)
Mongolism and dysautonomia	5 7 (6/6)	5 3 (6/6)	4 8 (6/6)	5 (6/6)	3 2 (6/6)	5 (6/6)	0 5 (1/6)	0 5 (1/4)	0 0	0 3 (1/6)	0 7 (3/6)	1 3 (4 6)

[a] *Abbreviations* ALA – alpha lactalbumin BLG – beta lactoglobulin
BSA – bovine serum albumin BGG – bovine gamma globulin
[b] Mean tube number and incidence of positive reactions are given Tube number refers to last test tube showing positive results Successive test tubes contained two-fold serial dilutions tube 1 being 1 20
[c] Other diseases refers to children admitted to hospital with various acute and chronic conditions not associated with milk sensitivity

with other antigens (personal communication) and this is assumed to be non specific Our technique for radio immunoelectrophoresis was based on Yagi s method (25) Details of this and the double micro immunodiffusion test in these experiments have al ready been published (9) Goat antisera against hu man IgA and IgM were purchased from Hyland Lab oratories Los Angeles Calif Goat anti human IgG and goat anti human globulins were prepared in our laboratory The antisera to IgG IgA and IgM were tested against human serum by immuno electrophore sis and found to be monospecific

Due to contaminants in a few of the milk proteins especially alpha lactalbumin and beta lactoglobulin as shown by the hemagglutination inhibition test this reaction was found to be not completely specific The data obtained by the radio immunoelectrophore sis and precipitation tests on the other hand proved to be more specific as the impurities did not affect the e reactions

RESULTS

Passive hemagglutinating coproantibody titers in healthy controls are shown in Table 1 Cop roantibodies were found even at the age of 1 month infants under 1 month were not ex amined The incidence of coproantibodies was slightly less than serum antibodies in the same group of patients The highest titer was 1 160 All cases with antibodies to cow s milk also had antibodies to one or more of the isolated proteins On the other hand not all individuals with antibodies to the isolated proteins had antibodies to whole milk The incidence of pos itive reactions in children under 1 year suffer ing from a variety of acute and chronic condi

Table 2 *Hemagglutinating serum and coproantibodies to milk proteins (group 4)*

Group	No in group	Serum						Feces					
		Whole milk	ALA[a]	BLG	BSA	BGG	Casein	Whole milk	ALA	BLG	BSA	BGG	Casein
Milk allergy													
Prolonged exposure	7	4 1[b] (7/7)	5 4 (7/7)	5 (7/7)	5 3 (7/7)	4 7 (7/7)	4 (7/7)	3 3 (6/7)	4 (6/7)	3 1 (5/7)	2 (3/7)	4 (6/7)	3 3 (5/7)
Short exposure	5	2 (4/5)	2 2 (4/5)	3 (4/5)	2 8 (4/5)	2 (4/5)	2 4 (4/5)	0 2 (1/5)	0 (0)	0 4 (4/5)	0 2 (1/5)	0 (0)	0 2 (1/5)
Celiac disease	4	3 7 (4/4)	4 2 (4/4)	4 5 (4/4)	4 (4/4)	3 2 (4/4)	3 7 (4/4)	1 7 (2/4)	3 (3/4)	2 (2/4)	2 (2/4)	1 7 (3/4)	3 (2/4)

[a b] See first two foot notes to Table 1

Table 3 *Incidence of precipitating serum and coproantibodies to milk proteins (all groups)*

	Serum						Feces					
	Whole milk	ALA	BLG	BSA	BGG	Casein	Whole milk	ALA	BLG	BSA	BGG	Casein
Healthy controls	4/104	6/104	3/104	0/104	4/104	0/104	0/21	2/21	0/21	0/21	0/21	0/21
Other diseases[b]	2/14	3/14	1/14	2/14	1/14	0/14	0/34	2/34	0/34	0/34	0/34	0/34
Mongolism and dysautonomia	5/6	5/6	2/6	0/6	2/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
Milk allergy												
Prolonged exposure	5/7	5/7	3/7	2/7	4/7	0/7	3/7	2/7	2/7	1/7	1/7	0/7
Short exposure	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Celiac disease	3/4	2/4	2/4	1/4	1/4	2/4	1/4	1/4	1/4	0/4	0/4	0/4

[b] See first and last foot notes in Table 1
Numbers indi ate positive reactions / total number of children examined

tions did not vary significantly from that in healthy controls Over the age of 1 year copro-antibodies were found *in only one out of ten* ıntrol cases tested

The children with mongolism and with dys ıtonomia were analysed together as their im unological reactions were similar and we ınsidered that aspiration of milk is a common ıctor in both The incidence of coproantibod 's *was small* while the incidence and the levels f antibodies in erum were abnormally high

Table 2 describes hemagglutination reac ons in milk allergy and celiac disease When xposure to milk has been sustained for over ' weeks *high* levels of antibodies are found in erum and *feces in* all cases When exposure o milk did not exceed a few feeds the anti ody re ponse did not differ markedly from hat of normal controls In celiac disease mean antibody titers in serum and feces tended to be rather higher than in *controls We could find* no evidence in any group that serum and cop roantibodies were *consistently directed against* the ame antigens Furthermore antibody re sponse gave no indication *which of the milk* proteins was responsible for symptoms of milk allergy

The general pattern of the hemagglutination reactions is also reflected by our studies of serum and coproantibodies by precipitation in gel (Table 3) The very high incidence of serum antibodies to milk in mongolism and dysautonomia is contrasted by the absence of copro-antibodies in these conditions On the other hand in gastrointestinal milk allergy prolonged exposure to milk *resulted in a raised incidence* of precipitating antibodies in feces and serum This correlation between serum and coproanti bodies held true for celiac disease as well

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A similar pattern prevailed also in mongo-lism and dysautonomia and was quite *different* from that obtained in milk allergy and celiac disease Although in the last two conditions erum antibodies were also mainly of the IgG class it was only here that serum milk anti bodies belonging to the IgA class could be found Coproantibodies to milk were the *rule* among milk sensitive children after prolonged exposure There were derived mainly from IgA and to a lesser extent from IgG

The similarity of immunoglobulins in serum and feces was proven by immunodiffusion and

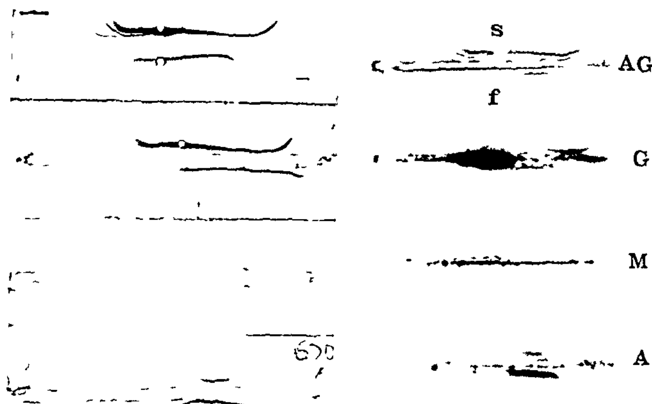


Fig 1 On the left are shown radio immunoelectrophoresis and on the right autoradiographs of serum (*s* upper well) and feces (*f* lower well) of an infant aged 3 months sensitive to cow's milk proteins To upper trough (*AG*) anti human globulin was added to the other troughs monospecific antihuman IgG IgM and IgA (slides *G* *M* and *A* respectively) Slides *AG* show that immunoglobulin and milk antibodies in serum belong mainly to IgG and in feces to IgA This is confirmed by slides *G* and *A* Very little immunoglobulin *M* was found in feces and no antibodies Note the similarity of immunoglobulins in serum and feces

lysis (Fig 3 top) All lines of the three fecal supernatants on the immunodiffusion slide showed lines of identity with IgG in the serum Two of these were identical with the IgA and one with IgM in the serum In fecal supernatants an additional line appeared which bound

Table 4 *Radio immunoelectrophoresis showing immunoglobulin class of milk antibodies*

Group	Serum			Feces		
	IgG	IgA	IgM	IgG	IgA	IgM
Healthy controls and children with other diseases [b]	1 6[a] (9/12)	0 (0/12)	0 (0/12)	0 (0/12)	0 1 (1/12)	0 (0/12)
Mongolism and dysautonomia	3 2 (4/4)	0 (0/4)	0 (0/4)	0 (0/4)	0 (0/4)	0 (0/4)
Celiac disease	2 6 (3/3)	0 6 (2/3)	0 (0/3)	0 (0/3)	1 (1/3)	0 (0/3)
Milk allergy						
Prolonged exposure	3 2 (5/5)	1 6 (3/5)	0 (0/5)	1 (3/5)	2 4 (4/5)	0 2 (1/5)
Short exposure	1 5 (2/2)	0 5 (1/2)	0 (0/2)	0 (0/3)	0 (0/3)	0 (0/3)

[a] Numbers refer to intensity of reaction with an arbitrary scale of 0–5 Mean and incidenc are given
[b] See last foot note to Table I

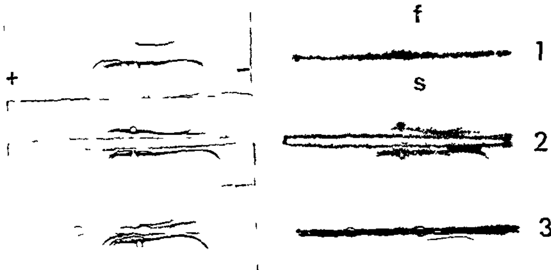


Fig 2 On the left are shown immunoelectrophoretic patterns of *1* normal child *2* child with milk sensitivity and *3* child with mongolism Upper well (*f*) fecal extract and lower well (*s*) serum from same individual The trough was filled with goat anti human serum globulin and radio-iodinated milk On the right radio-immunoelectrophoretic pattern of same individuals Note presence of IgA and IgG antibodies to milk in serum and feces of case 2 IgG predominating in serum and IgA in feces In case 3 only serum IgG antibodies are pres nt.

antigen thus making the radio-immunodiffusion method (as distinct from the radio-immunoelectrophoresis) unsuitable for detecting coproantibodies These lines were also observed by Kraft et al (18) Like others we found on immunoelectrophoresis of feces a substance running towards the anode (22) and this precipitate binds radio-activity It is possible that it is this substance which we found contaminating the precipitates in the radio-immunodiffusion reactions

DISCUSSION

The presence of hemagglutinating antibodies in feces to milk proteins is a physiological phenomenon (2) We found them to appear within a month after feeding of cow s milk. They were present in three quarters of normal infants and their incidence dropped after the age of 1 year Similar findings have been observed in the serum (8)

The exact period required for the appearance of coproantibodies in our cases and whether they precede serum antibodies is not known Observations with oral cholera vaccine suggest that coproantibodies appear earlier and disappear more rapidly (4 10) It appears that any food protein in the gastrointestinal tract may evoke an immunological response just as do pathogenic organisms The presence of precipitating or high levels of hemagglutinating antibodies is usually of pathological significance in the serum it may denote milk sensitivity celiac disease or aspiration (6) In feces it has previously been found only in milk and gluten sensitive infants (14 17) Both hemagglutination and precipitation in gel techniques produced a similar pattern of response in our groups of patients

Although we found abnormally high levels of antibodies in various pathological conditions we do not claim that these are sensitising antibodies and as such responsible for the clinical picture They may be blocking antibodies and thus reduce the severity of the reaction

The presence of coproantibodies doe not seem to be related to the presence of milk intake at the time of the test Two of our infants who had been off cow s milk for pro-

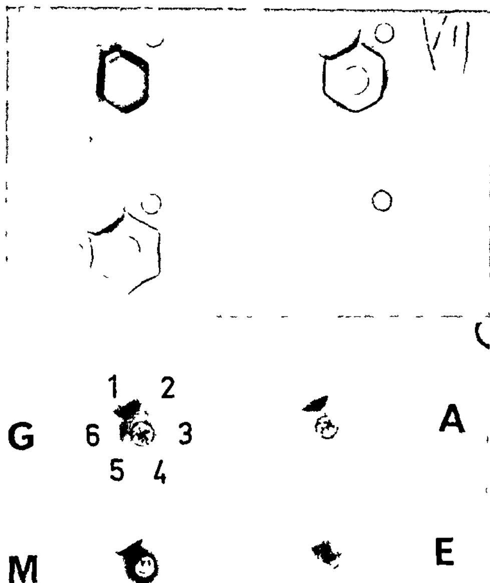


Fig 3 RID reactions of fecal supernatants (*1 3 5*) and sera (*2 4 6*) from three normal infants with goat antisera against IgG (*G*) IgM (*M*) IgA (*A*) and IgE (*E* diluted 1 30) Top the immunodiffusion slide bottom the corresponding autoradiograph Iodinated cows milk was added to the wells of the antisera Note the intensive false positive antigen binding on the precipitation lines of the fecal supernatants not related to immunoglobulins

longed periods still had coproantibodies On the other hand two infants who were just beginning to tolerate cow s milk again had none Most of the infants sensitive to milk in this study reacted clinically to beta lactoglobulin but not to the other antigens given by mouth (9) This sensitivity is not reflected in the serum and coproantibody titers which was high to a number of antigens High titers of antibodies to antigens to which the patient is not clinically sensitive may be a due to damage of the intestinal wall, allowing other antigens to enter the blood stream and producing a response Alternately they may denote a state of increased immunological responsiveness in the gastrointestinal tract

Abnormal levels of coproantibodies in our series were always associated with raised milk antibody titers in serum In mongolism and dysautonomia serum levels were high despite normal levels of coproantibodies It has been our contention (6) that in these two conditions aspiration of milk is the cause of high titers of milk antibodies

Antibodies may therefore be found in the serum and would be expected in the lungs but not in the gastrointestinal tract

Serum antibodies of IgA type were found by radio-immunoelectrophoresis only in milk sensitivity and celiac di ea e Although by radio-immunodiffusion we have found such antibodies even in healthy individuals their detection by the pre ent method seems to denote pathology The fact that we found coproantibodies to belong mainly to immunoglobulin A is in accordance with present concepts IgA is the major immunoglobulin of the gastrointestinal mucosa (5 7 11) and is the main constituent of antibodies after local sensitisation (12)

SUMMARY

An attempt has been made to delineate further the significance of milk coproantibodies The presence of precipitating and of high levels of hemagglutinating antibodies in feces was found only in patients with milk sensitivity or celiac disease These ca es showed also high levels of serum antibodies to milk In mongolism and dysautonomia no abnormal titers of coproantibodies were found in spite of strong reactions in the sera

Antibodies to milk were found by radio-immunoelectrophoresis to consist mainly of immunoglobulin A in feces and immunoglobulin G in serum Serum milk antibodies of the IgA type were found only in patients with milk sensitivity or celiac disease

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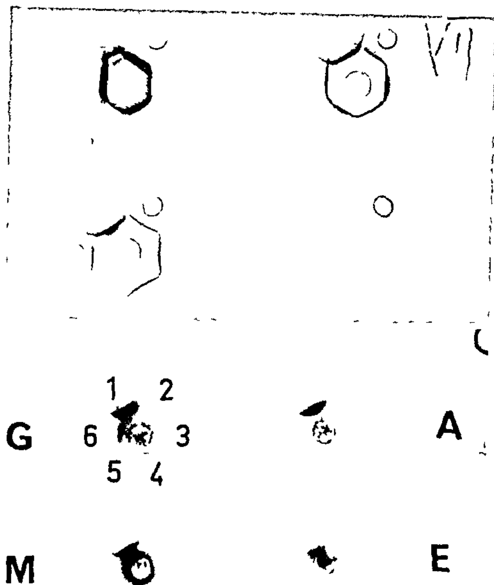


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CONTROL OF RESPIRATION IN NEWBORN BABIES

II *The Development of the Thoracic Reflex Response to an Added Respiratory Load*

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In a recent publication (2) it was shown that the strength of the Hering Breuer inflation reflex in babies (i e inhibition of inspiration in response to lung distension 3) varies with age being very weak at a postmenstrual age of 32 weeks to increase to a maximum strength at 36 to 38 weeks and then to decrease in strength again Whereas the primary weakness of the reflex at an early postmenstrual age *may* simply imply that some of its linkages fail at that stage the decreasing strength seen after 38 weeks is more difficult to explain Hypothetically the reason for this decline might be that other reflexes such as e g the thoracic spinal reflexes related to breathing then are becoming stronger and thus relatively more important

The existence of thoracic spinal reflexes in the nervous control of breathing has recently been demonstrated Campbell (4) found that an increase of the respiratory load leads to an increase of the mechanical power generation of the respiratory muscles and Sears (see 10) and v Euler and co-workers (see 6) later showed that such a response is reflexly mediated via thoracic dorsal root afferents from intercostal muscle spindles The aim of the present investigation was to study this reflex system in babies of different postmenstrual ages by recording the effect on the intrapleural pressure swings of an added respiratory load

MATERIAL AND METHODS

Eight normal" infants of varying gestational age were studied and the experiments were performed at varying times postnatally corresponding to 30½ to 42 weeks of postmenstrual age (Table 1) Four of the babies were studied more than once (Table 1) The calculation of the postmenstrual age (the sum of gestational and postnatal ages) was based on the mothers last normal menstruation and the length and weight of the baby at birth There was a good correlation between the postmenstrual age and the neurological behaviour and reflex pattern (8) in all the babies studied

An estimation of the capacity of the thoracic reflex system was achieved by recording the amplitude change of the intraesophageal pressure swings caused by occlusion of the airways Since the intraesophageal pressure equals the intrapleural pressure (7) a gradual change in amplitude of the former caused by airway occlusion can be assumed to reflect a change in the power generation of the respiratory muscles The intraesophageal pressure was recorded via an open ended saline filled polyetylene catheter introduced via a nostril to the lower third of the esophagus and connected to a pressure transducer (T Ljungstrom Sweden) The pressure swings were displayed on a Mingograph Recorder (Elema Schonander Sweden) A rubber face mask (no 0 Rendall Baker) slightly modified for the pressure recording was placed appropriately and sealed around the mouth and nose of the baby By simply occluding the opening of the mask a sudden increase of the respiratory load was achieved The occlusions were undertaken from the height of expiration or inspiration and only when the baby was breathing calmly When the occlusion was made at the height of an inspiration the Hering Breuer inflation reflex was of course also evoked (cf 2)

The rectal temperature was controlled throughout the experiments and found to remain constant at

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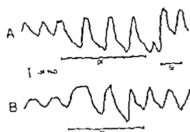


Fig 3 Tracings of the intraesophageal pressure swings during spontaneous breathing and airway occlusion (i e occlusion of the opening of the rubber face mask) in a baby of a postmentrual age of 37 weeks In A airway occlusion begins at the height of an expiration in B at the height of an inspiration Inspiration deflection downwards

an inspiration In both cases the intraesophageal pressure amplitude decreases as a consequence of the occlusion Moreover the respiratory rate increases slightly although in B where the occlusion is performed at the height of an inspiration a slight prolongation of the first consecutive expiratory phase is seen (Hering Breuer inflation reflex cf 2) Fig 2 shows the corresponding tracings from three other babies 31 (A) and 32 (B C) weeks of age and illustrates the variability of the changes in re*spiratory rate* in response to airway occlusion at this developmental stage In all cases the occlusion is performed at the height of an expiration and as can be seen the amplitude of the pressure swings decreases as a consequence of the occlusion However in the first case (A) a few shallow breathes is followed by complete respiratory arrest lasting until the face mask is removed In the second case (B) a rapid fibrillating breathing pattern occurs and in the third case (C) breathing becomes highly irregular Fig 3 finally shows the respiratory responses to airway occlusion in a 37 weeks old baby Both when the occlusion is performed at the height of an expiration (A) and at the height of an inspiration (B) a pronounced gradual increase of the amplitude of the pressure swings takes place This response is similar to that of adult animals and man which reflects an increasing power generation of the respiratory muscles in response to the added load (4 6 9) There is also a decrease in respiratory rate which is more pronounced in B where the Hering Breuer inflation reflex is also evoked (cf 2)

The change with age of the respiratory muscle response to an added load has been illustrated graphically in Fig 4 Here the relative increase in amplitude caused by the occlusion has been plotted against the postmenstrual age of the baby at the time of the experiment Except for a decrease between 34 and 37 weeks the response is seen to increase gradually in strength between 30 and 42 weeks of age As can be

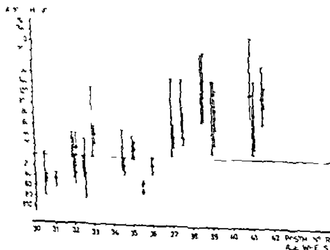


Fig 4 Diagram showing the strength of the response to airway occlusion (ordinate) in 8 babies studied at 19 different occasions plotted against the postmenstrual age of the babies at the time of the experiment (abscissa) The strength of the response to airway occlusion has been represented as the mean relative change in amplitude of the intra esophageal pressure swings of the 3 (occasionally 2) first breathing cycles during occlusion as compared to the mean amplitude of 5 breathing cycles immediately preceeding the occlusion The filled circle derive from experiments where the occlusion has been undertaken from the height of an expiration and the open circles from experiments where the occlusion has been undertaken from the height of an inspiration The vertical lines represent the ranges

Table I *Data of infants examined*

Case	Birth weight (g)	Length at birth (cms)	Gestational age (weeks)	Postmenstrual age at experiment (weeks)
I female	1 420	40	31–32	32–33 34–35 35–36 37–38
II female	3 220	49	37	38–39
III male	1 630	39	30	30–31 31 32 35–36
IV male	4 420	52	40	41
V male	3 230	52	37	37
VI female	1 030	37	30	32 33 35 36 39 41
VII female	3 500	49	39	39
VIII male	3 160	51	42	42

36–37 C The smaller babies were studied in incubators Since the face mask adds to the dead space of the airways it was kept to the face for only a few minutes each time

RESULTS

It was soon found out that the respiratory response to an added load varied with the postmenstrual age of the baby Thus in the youngest babies studied the amplitude of the intraesophageal pressure swings remained constant or even decreased on airway occlusion whereas in the older babies the pressure amplitude increased instead this increase being larger the older the baby Moreover the older the baby the more constant was the change in respiratory rate caused by airway occlusion These findings are illustrated in Figs 1–3

Fig 1 shows the tracings of the intraesophageal pressure swings during spontaneous breathing and the effect of airway occlusion in a 32 weeks old baby In A occlusion of the opening of the face mask is performed at the height of an expiration in B at the height of

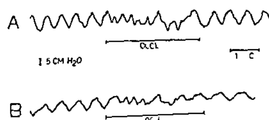


Fig 1 Tracings of the intraesophageal pressure swings during spontaneous breathing and airway occlusion (i e occlusion of the opening of the rubber face mask) in a baby of a postmenstrual age of 32 weeks In A airway occlusion begins at the height of an expiration in B at the height of an inspiration Inspiration deflection downwards

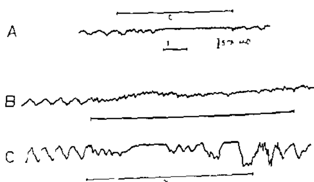


Fig 2 Tracings of the intraesophageal pressure swings during spontaneous breathing and airway occlusion from the height of an expiration) in three babies 31 (A) and 32 (B C) weeks of postmenstrual age Inspiration deflection downwards

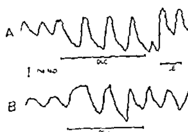


Fig 3 Tracings of the intraesophageal pressure swing during spontaneous breathing and airway occlusion (i e occlusion of the opening of the rubber face mask) in a baby of a postmenstrual age of 37 weeks In A airway occlusion begins at the height of an expiration in B at the height of an inspiration Inspiration deflection downwards

an inspiration In both cases the intraesophageal pressure amplitude decreases as a consequence of the occlusion Moreover the respiratory rate increases slightly although in B where the occlusion is performed at the height of an inspiration a slight prolongation of the first consecutive expiratory phase is seen (Hering Breuer inflation reflex cf 2) Fig 2 shows the corresponding tracings from three other babies 31 (A) and 32 (B C) weeks of age and illustrates the variability of the changes in respiratory rate in response to airway occlusion at this developmental stage In all cases the occlusion is performed at the height of an expiration and as can be seen the amplitude of the pressure swings decreases as a consequence of the occlusion However in the first case (A) a few shallow breathes is followed by complete respiratory arrest lasting until the face mask is removed In the second case (B) a rapid fibrillating breathing pattern occurs and in the third case (C) breathing becomes highly irregular Fig. 3 finally shows the respiratory responses to airway occlusion in a 37 weeks old baby Both when the occlusion is performed at the height of an expiration (A) and at the height of an inspiration (B) a pronounced gradual increase of the amplitude of the pressure swings takes place This response is similar to that of adult animals and man which reflects an increasing power generation of the respiratory muscles in response to the added load (4 6 9) There is also a decrease in respiratory rate which is more pronounced in B where the Hering Breuer inflation reflex is also evoked (cf 2)

The change with age of the respiratory muscle response to an added load has been illustrated graphically in Fig 4 Here the relative increase in amplitude caused by the occlusion has been plotted against the postmenstrual age of the baby at the time of the experiment Except for a decrease between 34 and 37 weeks the response is seen to increase gradually in strength between 30 and 42 weeks of age As can be

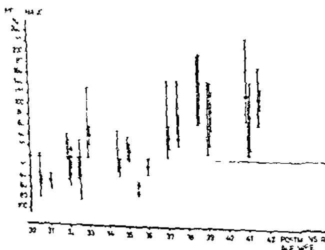


Fig 4 Diagram showing the strength of the response to airway occlusion (ordinate) in 8 babies studied at 19 different occasions plotted against the postmenstrual age of the babies at the time of the experiment (abscissa) The strength of the response to airway occlusion has been represented as the mean relative change in amplitude of the intraesophageal pressure swings of the 3 (occasionally 2) first breathing cycles during occlusion as compared to the mean amplitude of 5 breathing cycles immediately preceeding the occlusion The filled circles derive from experiments where the occlusion has been undertaken from the height of an expiration and the open circles from experiments where the occlusion has been undertaken from the height of an inspiration The vertical lines represent the ranges

Table 1 *Data of infants examined*

Case	Birth weight (g)	Length at birth (cms)	Gestational age (weeks)	Postmenstrual age at experiment (weeks)
I female	1 420	40	31–32	32–33 34–35 35–36 37–38
II female	3 220	49	37	38–39
III male	1 630	39	30	30–31 31 32 35–36
IV male	4 420	52	40	41
V male	3 230	52	37	37
VI female	1 030	37	30	32 33 35 36 39 41
VII female	3 500	49	39	39
VIII male	3 160	51	42	42

36–37 C The smaller babies were studied in incubators Since the face mask adds to the dead space of the airways it was kept to the face for only a few minutes each time

RESULTS

It was soon found out that the respiratory response to an added load varied with the postmenstrual age of the baby Thus, in the youngest babies studied, the amplitude of the intraesophageal pressure swings remained constant or even decreased on airway occlusion whereas in the older babies the pressure amplitude increased instead this increase being larger the older the baby Moreover the older the baby, the more constant was the change in respiratory rate caused by airway occlusion These findings are illustrated in Figs 1–3

Fig 1 shows the tracings of the intraesophageal pressure swings during spontaneous breathing and the effect of airway occlusion in a 32 weeks old baby In A occlusion of the opening of the face mask is performed at the height of an expiration in B at the height of

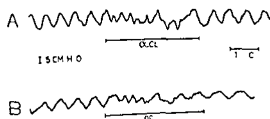


Fig 1 Tracings of the intraesophageal pressure swings during spontaneous breathing and airway occlusion (i e occlusion of the opening of the rubber face mask) in a baby of a postmenstrual age of 32 weeks In A airway occlusion begins at the height of an expiration in B at the height of an inspiration Inspiration deflection downwards

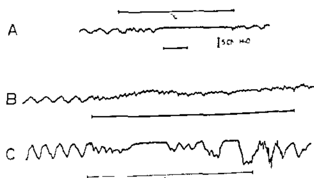


Fig 2 Tracings of the intraesophageal pressure swings during spontaneous breathing and airway occlusion (from the height of an expiration) in three babies 31 (A) and 32 (B C) weeks of postmenstrual age Inspiration deflection downwards

from the height of an expiration when the pulmonary tension receptors are thus differently stimulated which further indicates that the response in the older babies is not evoked via pulmonary vagal afferents Also hypercapnia and hypoxia as the cause of the increase in amplitude of the first few breaths after occlusion seems unlikely as pointed out by earlier authors (4)

A similar development of the response to an added respiratory load as described in the present work for newborn babies has been shown to occur postnatally in kittens (9) In such animals the development of the respiratory muscle response to airway occlusion has been tentatively related to the development of the nerve fibres and especially to the innervation of the intercostal muscle spindles (9) The present finding in the youngest babies of a decreas of the amplitude of the intraesophageal pressure swings in response to airway occlusion (Figs 1 and 2) indicates that, the addition of a respiratory load inhibits the activity of the respiratory muscles at that stage Such an inhibition has also been demonstrated in newly born kittens and again related to the innervation of the intercostal muscle spindles (9) Thus the findings can be explained if the intercostal muscle spindles—like hind leg muscle spindles of newborn kittens (see 11)—lack an efferent "gamma innervation at that stage Then the spindles function as passive stretch receptors only and will not respond to an added load Instead the inhibitory influence from the Golgi tendon organs might dominate thus explaining the finding of an inhibition of the muscle activity in response to the airway occlusion (cf 9)

In an earlier publication (2) it was shown that the Hering Breuer inflation reflex is very weak in babies 32–34 weeks of postmenstrual age This finding was principally confirmed in the present investigation (Figs 1 B and 5) However the fact that occlusion in maximal expiration (which prevents lung expansion and activation of the Hering Breuer inflation reflex) at that stage produced an irregular breathing pattern and sometimes respiratory arrest (Fig 2) still shows that this reflex although weak is of primary importance for a proper functioning of the respiratory center as is the case in newborn animals (9)

An interesting observation is that the development of the strength of the thoracic reflex to an added load shows a dip around 36–38 weeks or when the Hering Breuer reflex reaches its maximum strength (Figs 4 and 5) This might indicate that the two reflexes in some way compete Nothing seems to be known about the influence from the intercostal and thoracic wall receptors on supraspinal centers or of a possible competition between thoracic and pulmonary reflexes but if such an interaction does exist, this could also explain why the Hering Breuer inflation reflex decreases in strength after 38 weeks of age when the thoracic reflex continues to increase in strength

SUMMARY

The respiratory response to an added load recorded as the change in amplitude of the intraesophageal pressure swings caused by airway occlusion has been studied in 8 babies $30\frac{1}{2}$ to 42 weeks of postmenstrual age With increasing age the response to such an occlusion was found to increase gradually and it is concluded that this is a reflection of an increasing maturation of a thoracic respiratory reflex The results are related to similar findings in developing animals and to earlier studies on the development of the Hering Breuer inflation reflex in babies

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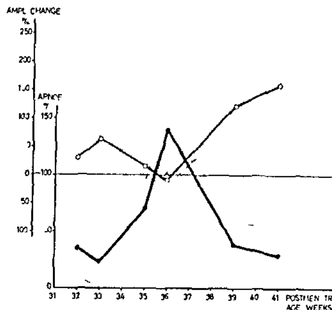


Fig 5 Diagram showing the development of the strength of the Hering Breuer inflation reflex (filled circles) and of the strength of the thoracic reflex response to an added load (open circles) in one and the same baby studied at six occasions between 32 and 41 weeks of postmenstrual age The strength of the Hering Breuer inflation reflex was calculated as the relative increase in length of the first breathing cycle following airway occlusion from the height of a normal inspiration (see Ref 1) The strength of the thoracic reflex response was calculated as the mean relative increase in amplitude of the intraoesophageal pressure swings of the first 3 breaths following airway occlusion from the height of a normal expiration Shaded areas represent ranges

seen, in this respect the results are the same irrespective of whether occlusion was performed from the height of an expiration (filled circles) or an inspiration (open circles) Since the response in the older babies is principally the same as in adult animals and man where it has been attributed to a thoracic spinal reflex (4, 6 9) it may be concluded that the strength of this reflex increases with postmenstrual age of the baby

In one baby estimations both of the strength of the Hering Breuer inflation reflex and of the strength of the amplitude response to an added load i e of the thoracic reflex were made at different occasions between 32 and 41 weeks of postmenstrual age The results plotted in Fig 5, show the same principal development of the reflexes in this single baby as the plottings of all individual values (see Fig 3 and Ref 2)

It may be pointed out that here also the development of the amplitude response shows a dip around 36 weeks or when the Hering Breuer inflation reflex reaches its maximum strength

DISCUSSION

In adult animals and man airway occlusion is known to provoke reflexes both from the lungs via the vagus nerves and from the thoracic wall via the intercostal nerves (3 4) Whereas the vagal reflexes seem to affect mainly the respiratory rate (3, 12) the thoracic reflexes have been shown to affect the power generation of the respiratory muscles (4 6 10) The present finding of an increase with age of the power generation of the respiratory muscles in response to airway occlusion (Figs 1 3 4 and 5) thus indicates that the importance of the thoracic reflex system increases with postmenstrual age It may be argued that since the face mask (volume 10 ml) used was the same for all babies a relatively larger dead space and thus a smaller elastic load was added to the smallest babies which may then explain the feeble response in these individuals Since however the elastic load produced by airway occlusion is not related to the dead space only but rather to the total lung volume which was probably less than 100 ml in the smallest babies and more than 300 ml in the biggest ones (cf 1 and 5), the added load must rather have decreased with age instead Another argument might be that the amplitude response is a consequence of an altered vagal afference due to the airway occlusion Thus when the airways are occluded from the height of expiration the lungs are prevented from expansion during the next inspiratory effort so the Hering Breuer inhibition reflex is not activated Although this explains the prolonged durations of the inspiratory phases in the older babies (Fig 3) it does not explain the gradual increase in intraesophageal pressure amplitude Moreover the present results show that the amplitude response was essentially the same when the occlusion was performed from the height of an inspiration as

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THE COMBINED XYLOSE DISACCHARIDE TOLERANCE TEST ITS APPLICATION FOR DIAGNOSING DISACCHARIDASE DEFICIENCY

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The main cause of a flat blood glucose curve after ingestion of a disaccharide e g lactose or sucrose lies in defective digestive absorptive function (6) low gastric or intestinal motility probably being of less importance (1) If we wish to differentiate a disaccharidase deficiency from other causes of defective sugar absorption at least two tests e g a disaccharide test and a test with its component monosaccharides are needed (4 11 21)

We felt that the disaccharide test might be improved by administering the disaccharide in combination with the pentose *d*(+) xylose Xylose absorption in adults and children is well documented and the xylose test is often used in diagnosing malabsorption (2 3 5 12 14 15 16) By measuring simultaneously xylose and disaccharide absorption it should be possible to assess the digestion of the tested disaccharide in a single test because xylose being absorbed by the same mechanism as other sugars with the pyranose ring structure such as glucose and galactose (19) would serve as an indicator for the absorptive function

In this article the results of combined xylose disaccharide tolerance tests in children with sucrase isomaltase deficiency are described For comparison the results of the same tests in normal children are given Our experience with the xylose test is discussed briefly

MATERIAL AND METHODS

The study comprised two groups of children (I) 61 normals" and (II) six children with a sucrase isomaltase deficiency The normal children were a heterogeneous group including some children with a history of diarrhoea Not included were children with proven villous atrophy of jejunal mucosa, disaccharidase deficiency liver disorders or inborn errors of metabolism The diagnosis sucrase isomaltase deficiency of the second group had been confirmed by enzymic assay in a bioptic sample of jejunal mucosa the pertinent data are given in Table 1 The patients were biopsied with the twin hole capsule of Sebus (20) No medication was given during the experiments *d*(+)-Xylose was administered alone or combined with glucose fructose lactose *maltose* sucrose or starch The doses used were 0.5 g xylose and 2 g of the other sugars per kilogram body weight with a maximum of 25 and 50 g respectively The required amount of xylose was dissolved in a 10% solution of the test sugar Thus the xylose concentration was 2.5% at a total dose of 12.5 g or lower and 5% at the maximum total dose of 25 g xylose and 50 g other" sugar in 500 ml water After ingestion of the sugar solution capillary blood samples were taken at 30 min intervals during 3 hours No attention was given to the position or the activity of the child The *fasting period* prior to the test was usually 14 hours infants were *fasted for* at least 8 hours Blood xylose was estimated according to Roe & Rice (18) Blood glucose was estimated with glucose oxidase (reagent set TC M of Boehringer Mannheim Germany)

RESULTS

Fig 1 shows the mean blood xylose levels (±2 S D) in 90 oral tolerance tests performed

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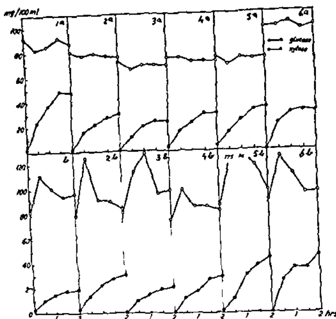


Fig 3 Xylose sucrose tolerance test (1 *a*–6 *a*) and xylose lactose tolerance test (1 *b*–6 *b*) in six children with sucrase isomaltase deficiency Nos 1 and 2 are brothers The last hour of the test has been omitted since it yields no relevant information

there are considerable variations in xylose levels during the tests (Fig 1) the xylose curves of each child (Fig 2) are much less divergent This confirms the good reproducibility of the test as reported by Brien et al (3) Incidentally the data indicate that the addition of another sugar to the xylose test has only a relatively small competitive effect on xylose levels Likewise when xylose was combined with starch boiled in water (10 % solution) we found the ensuing xylose curve to be only slightly affected (experiments on three children) Benson (2) found a distinct competition effect when he added xylose to a meal For the present purpose a competition effect if present is of little consequence since xylose absorption would be enhanced rather than depressed by a decreased glucose release from the ingested disaccharide

Beck et al (1) did not find any influence of a prolonged gastric emptying time on xylose levels except with complete or almost complete pyloric obstruction This agrees with our findings we observed abnormally low xylose levels only in two *infants* with pyloric stenosis the xylose curves of three other children with proven delayed emptying of the stomach but normal absorption being essentially normal A flat xylose curve after xylose loading is almost always caused by a substantially reduced absorptive function (5 12 14 15) We found significantly low xylose levels in three out of six celiac children

The xylose curves of the sucrase isomaltase deficient children were all in the normal range The flat glucose curves after sucrose loading are in marked contrast with the normal xylose rise (Fig 3) From the distinct xylose rise one must conclude that the glucose absorption mechanism is intact So the flat glucose curve must be due to impaired sucrose hydrolysis i e to low sucrase activity Lactase activity was normal as follows from the result of the combined xylose lactose test in the same children

Thus the combined xylose disaccharide test provides a dependable means to diagnose in a single test a deficiency of the disaccharidase concerned It also allows a more certain interpretation of the disaccharide test as the latter is sometimes inconclusive and may even be at variance with the disaccharidase activity of biopsy specimens (10 17)

We derive the following interpretation of the test from our experiments (1) a normal rise of

Table 1 *Data of six children with disaccharidase deficiencies*

			Enzyme activities[a] in jejunal mucosa				
Patient	Sex	Age	Sucrase	Isomaltase	Palatinase	Maltase	Lactase
R T	♂	7	1 9	—	0 2	6 0	4 3
A T	♂	5	0 0	—	0 0	7 3	4 3
C R	♂	2	0 0	0 5	—	3 6	3 5
M S	♂	1	0 0	0 9	—	4 1	7 0
W E	♀	8	0 3	—	0 2	1 5	9 6
J K	♂	14	0 1	0 9	—	4 8	6 2
Normal			4 0–17 4[b]	3 8–16 8[b]	1 5–4 0[c]	15 8–55 6[b]	1 3–11 8[b]

[a] μ Moles substrate hydrolyzed per minute and per gram tissue (palatinase method of Dahlquist (7) other disaccharidases method of Eggermont (8)
[b] Range of a control group of 35 children at ages from 1 month up to 14 years Data from Eggermont & Hers (9)
[c] Data from Dahlquist (7)

on the control groups of 61 children Fig 2 shows the results of both a xylose test and a combined xylose glucose or xylose-sucrose test performed on four children of the control group For each child the xylose curves, whether combined with another sugar or not, were more similar than the curves of different children For greater clarity the glucose data have been omitted from the Fig 2, but in all combined tests glucose rose normally The results of the combined xylose disaccharide tests performed on six children with sucrase isomaltase deficiency are presented in Fig 3 When xylose was combined with sucrose the xylose level rose and the glucose level remained constant (Fig 3 upper part) but simultaneous oral administration of xylose and lactose resulted in a concomitant rise of both xylose and glucose (Fig 3 lower part)

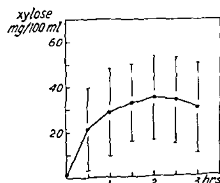


Fig 1 Mean blood xylose levels (±2 S D) of 90 oral tolerance tests performed on 61 normal children Xylose was administered alone or combined with glucose fructose sucrose or starch

DISCUSSION

Our xylose dose was the same as used by Jones & Di Sant Agnese (13) and approximates the dose used by Ingomar et al (12), Meeuwisse & Dano (15), and Mollerberg & Soderhjelm (16) viz 15 g per square meter of body surface We did not observe any side effects of the test such as one or more loose stools except during the xylose sucrose test in the sucrase isomaltase deficient children Meeuwisse & Dano (15) who used a 10% xylose solution saw these also in other children

While among the control group consisting of children without apparent absorption defect

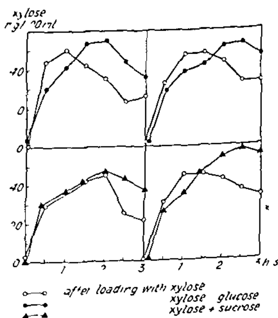


Fig 2 Xylose tolerance test in four normal children

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blood xylose in combination with a flat glucose curve (Fig 3) indicates a disaccharidase deficiency since the xylose rise renders a defective sugar absorption mechanism unlikely, (II) if both glucose and xylose rise normally, impaired hydrolysis of the tested sugar is excluded, (III) if the xylose rise is small, an impaired absorptive function is likely, though nothing can be said about its cause In our experience, the result of the combined xylose-disaccharide test is seldom ambiguous

In conclusion, we recommend the addition of xylose to the disaccharide tolerance rest if a child is suspected of having a disaccharidase deficiency

SUMMARY

Six children with a sucrase-isomaltase deficiency were subjected to an oral tolerance test with sucrose or lactose combined with $d(+)$ xylose

Simultaneous determination of a xylose curve allowed a more certain interpretation of the blood glucose curve A flat glucose curve combined with a normal xylose rise points to an impaired enzymic hydrolysis rather than to a defective absorption of its component monosaccharides Compared with the normal range ± 2 S D derived from 90 xylose curves of 61 normal children the sucrase isomaltase deficients showed normal xylose curves

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STABILITY OF ACID PHOSPHATASE OF FETAL RED BLOOD CELLS DURING INCUBATION WITH ACETYLPHENYLHYDRAZINE

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Maintenance of the sulfhydryl (SH) groups of erythrocyte components in the reduced state has been considered important for the functional and structural integrity for the normal survival of the red blood cell (8 9 10) It has been shown previously that during incubation with acetylphenylhydrazine (APH) or with oxidized glutathione (GSSG) the red blood cell acid phosphatase of adult subjects undergoes striking changes in its electrophoretic properties associated with a marked reduction in enzymatic activity (2–4) Since this enzyme is SH dependent, the investigation of its properties in the red blood cells (RBC) from new born infants could contribute to a better under standing of some aspects of RBC metabolism during this early period of life In particular it could help to define the relationship between the increased instability of reduced glutathione (GSH) and the high vulnerability of neo natal RBC to oxidative damage

In a previous study (5) we have shown that the acid phosphatase of fetal RBC is less stable than that of maternal RBC during incubation with APH The results of the present study confirm these observations and show a significant correlation between the fall in GSH concentration and the diminution in acid phosphatase activity in the red cells This study also shows that on the 3rd day of life the RBC acid phosphatase is more stable than at birth

MATERIAL AND METHODS

The blood samples for the study were obtained from 20 normal full term newborn infants and their mothers and from 13 normal premature infants (gestational age <35 weeks weight <2 3 kg) and their mothers Blood was withdrawn with plastic syringes from the umbilical vein at birth and in 10 infants also from the jugular or femoral vein at 3 days of age Blood from mothers was obtained from the antecubital vein shortly before or after delivery and in 10 of the same mothers also 3 days post partum All infants were Rh and ABO compatible with their mothers the direct Coombs test on cord blood was negative and serum bilirubin levels at 3 days of age were within normal limits The RBC of 8 normal adults (4 males and 4 females) were also studied

The blood was immediately transferred into heparinized test tubes mixed and centrifuged at 3 000 r p m for 10 min After centrifugation the plasma and buffy coat were removed and the RBC were washed 3 times with cold saline and then resuspended in saline to a final hematocrit of 50 % The suspensions of neonatal and maternal RBC with and without APH (4 mg ml of suspension) were then incubated aerobically and with continuous agitation for 2 hours at 37 C In 6 experiments the simultaneous changes in GSH concentration and acid phosphatase activity were followed during incubation by withdrawing small aliquots of RBC suspension at fixed time intervals (0 30 45 60 and 120 min)

Table 1 *Acid phosphatase activity of red blood cells from different subjects after incubation with acetylphenylhydrazine for 2 hours at 37 C*
The enzymatic activity has been expressed as percent of the activity before incubation

Group studied	No of subjects	Residual enzymatic activity (average ± S D)	% Difference	Significance of the difference[a]
Full term infants at birth	20	43 2 ± 8 6	27 8	$p < 0\ 001$
Mothers at delivery	20	55 2 ± 10 5		
Full term infants at 3 days	10	43 3 ± 10 0	20 9	$p < 0\ 001$
Mothers at 3 days post partum	10	52.3 ± 9 6		
Full term infants at birth	10	39 8 ± 8 1	8 6	$p < 0\ 05$
Full term infants at 3 days	10	43 3 ± 10 0		
Mothers at delivery	10	56 3 ± 10 6	7 0	Not significant
Mothers at 3 days post partum	10	52.3 ± 9 6		
Premature infants at birth	13	47 6 ± 11.5[b]	12.4	$p < 0\ 01$
Mothers at delivery	13	53 5 ± 13 3		

[a] The Student s t test was calculated for paired observations
[b] This value does not differ significantly from that of full term infants at birth

sured according to Beutler et al (1) and the acid sphatase activity was measured using a modifica of the method described by Torriani (10 11) 4 determinations of RBC acid phosphatase before after 2 hour incubation with APH were performed 3 normal adults Variance analysis of the results wed that the variability of the method was signifi tly lower than the variability among individual ects ($p < 0\ 001$) The data also showed that there e no appreciable differences in 2 consecutive asurements performed in the same subject at inter s of 8 days

RESULTS

ie results obtained in 20 full term and in premature infants and in their mothers are own in Table 1

The inactivation of RBC acid phosphatase ring incubation with APH was significantly eater in the erythrocytes of full term and remature infants than in maternal red cells $< 0\ 001$ and $< 0\ 01$ respectively)

In those infants studied also at 3 days of age he RBC acid phosphatase was significantly more stable than at birth ($p < 0\ 05$) However he enzyme was still significantly less stable han in maternal blood It should also be noted hat most of the infants studied at 3 days were among those who had showed a marked in stability of acid phosphatase at birth when compared with their mothers

The results obtained in mothers on the 3rd post partum day were not significantly different from those obtained in the same subjects at the time of delivery The acid phosphatase stability of RBC from premature infants at birth was not significantly different from that of RBC of full term newborns In Table 1 the residual acid phosphatase activity after incubation with APH has been expressed as per cent of the activity before incubation When RBC were incubated for 2 hours without APH there was a slight fall in acid phosphatase activity which was similar in maternal and fetal RBC (Fig 1) When the residual acid phosphatase activity after incubation with APH was expressed as per cent of the activity in the same RBC in cubated for 2 hours without APH the results did not change and the differences mentioned above were present to the same degree

Fig 1 shows the results of 6 experiments where the fall in GSH and in acid phosphatase activity were determined simultaneously in fetal and maternal RBC during incubation with and without APH The fall in acid phosphatase ac tivity followed with a slight delay that of GSH

both in maternal and fetal RBC The GSH level began to decrease earlier in fetal than in maternal RBC and the difference between the 2 bloods was already significant after 30 min of incubation Also the fall in acid phosphatase activity of fetal RBC preceded that of maternal RBC, however, the difference became significant only after 45 min of incubation

Fig 2 shows a clear correlation between the fall in GSH and that in acid phosphatase activity in both maternal and fetal RBC incubated with APH It is also evident that for a given per cent fall in GSH level the acid phosphatase activity decreased more in fetal than in maternal RBC

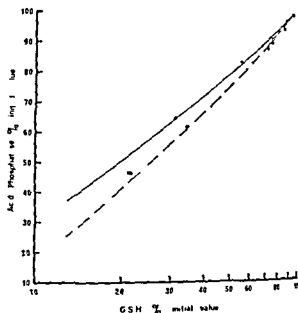


Fig 2 Relationship between the changes of GSH level and those of acid phosphatase activity (AP) in maternal (●) and fetal (○) RBC incubated with APH Regression equations Maternal RBC AP =Log GSH × 69 0–39 6 r = 0 96 Fetal RBC AP% = Log GSH° × 84 2–68 2 r = 0 97 The slopes of the two regression lines are significantly different (p < 0 05)

DISCUSSION

The acid phosphatase of human RBC presents a genetic polymorphism with 6 different electrophoretic phenotypes (6, 7) which show a different sensitivity to the action of oxidizing substances (12) We did not determine the electrophoretic phenotypes of RBC acid phosphatase in our cases and therefore we cannot be certain that the phenotypic distribution of maternal bloods was perfectly comparable to that of fetal and neonatal bloods However essentially the same differences between maternal and fetal blood were previously obtained in another laboratory under slightly different experimental conditions (5) Furthermore since the fetal genotype is half determined by the maternal genotype it seems unlikely that the phenotypic distribution of fetal bloods was very different from that of maternal bloods

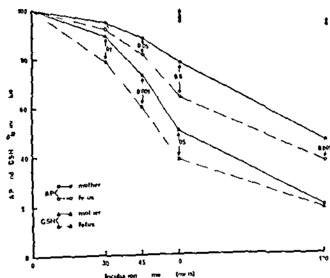


Fig 1 Changes in acid phosphatase activity (AP) and GSH concentration in maternal (● ▲—) and fetal (○ △) RBC incubated for 2 hours at 37 C with and without APH The significance of the difference at various intervals is also shown

Other authors have demonstrated a significant GSH instability in the RBC of newborn infants This is due in part to the physiologic hypoglycemia and in part to the metabolic features of the neonatal RBC (11 17) It is possible that GSH instability plays a role in the increased erythrocyte destruction which seems to take place during the first few days of life However at the present time this remains only a possibility and a speculation since the biochemical events immediately preceding RBC destruction are not known Our previous observations on the modifications induced by GSSG on RBC acid phosphatase (2–4) suggest

that the formation of mixed disulfides with GSSG could induce an inactivation of some enzymes and perhaps a modification of other structures in the RBC which could be important for the normal erythrocyte survival

Th role of acid phosphatase in RBC metabolism is not known but it is possible that modifications similar to those observed by us for this enzyme could also be induced in other more important SH dependent structures

The results of the present study indicate that inactivation of acid phosphatase in RBC incubated with APH (without glucose) is preceded by a fall in GSH and that the two events are significantly correlated It is therefore possible that the more rapid and marked fall in acid phosphatase activity of fetal RBC is the consequence of the increased GSH instability in these RBC when compared to the maternal erythrocytes The GSH content of fetal and neonatal red blood cells is higher than that of the adult (13 14) therefore when the same percentage of initial GSH is oxidized more GSSG is formed in the fetal than in the adult RBC This finding could explain why for a given percent fall of GSH in the RBC more acid phosphatase is inactivated in fetal than in maternal erythrocytes

The uptake of APH by fetal and maternal RBC was not measured in the present experiments Therefore the possibility that the observed differences between fetal and maternal RBC could be due at least in part to a different uptake of APH cannot be excluded

The increase in acid phosphatase stability from birth to 3 days of age suggests that the fetal RBC population is not homogeneous and that those RBC which are less capable of protecting *their SH* dependent structures from oxidation are destroyed soon after birth Some modifications of the internal milieu taking place early in extrauterine life such as a certain degree of hypoglycemia and an increased oxygen tension could favor this destruction and could contribute to the selection of a RBC population which is more stable in the changing biochemical environment

SUMMARY

Incubation of red blood cells (RBC) *in vitro* with acetylphenylhydrazine and without glucose produced an inactivation of acid phosphatase which was more marked in the fetal RBC than in the RBC from maternal blood The fall in reduced glutathione (GSH) during incubation was more rapid in fetal than in maternal RBC In each instance the fall in GSH preceded that in acid phosphatase and the two phenomena were very well correlated The faster inactivation of acid phosphatase in the fetal RBC could be due to the higher total concentration of GSH and to its decreased stability in fetal RBC

The stability of acid phosphatase seemed to increase in red blood cells from infants 3 days old This finding could indicate a certain degree of heterogeneity in the fetal RBC population at birth and a rapid selection in favor of those RBC which are more fit for the new biochemical environment after birth

ACKNOWLEDGEMENTS

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A NEW SYNDROME OF DYSMORPHOGENESIS IMPERFORATE ANUS ASSOCIATED WITH POLY OLIGODACTYLY AND SKELETAL (MAINLY VERTEBRAL) ANOMALIES

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Although imperforate anus is commonly associated with congenital malformations of the genitourinary and gastrointestinal systems skeletal anomalies including club foot dislocation of the hips and sacral deformities are also frequently seen (2 4 8) In 1968 a new syndrome of dysmorphogenesis was described in which certain skeletal malformations including polydactyly were associated with imperforate anus (5) In this preliminary report ten unrelated patients were presented Malformations of the vertebrae and the ribs were quite common in these patients while in some of them the iliac bones and the lower extremities were also involved

The similarity of these findings to those seen among the members of the luxoid group of mouse mutants especially such as the dominant hemimelia (Dh mutant) (6) was quite striking hence the possibility of a hereditary factor as a cause of this syndrome was considered Since these patients were ascertained through their polydactyly it was suggested that this syndrome may very well include forms of limb deformities other than polydactyly

During a retrospective study of 258 cases of imperforate anus seen at this hospital between 1957 and 1969 (inclusive) nine patients were found to have hand malformations This report describes the malformations encountered in these patients together with a review of the similar cases in the literature

CASE REPORTS

Case 1 O 4 day-old male was the second child of young parents. Physical examination additionally revealed polydactyly of th left hand and omphalocele No X rays of the spine were taken The father was 4 and the mother 23 years of age There was no consanguinity between the parents and no family history of congenital anomalies including polydactyly

Case 2 O H A 2-day-old male had an extra digit at right X rays of the spine revealed th vertebrae to be normal The left iliac bone was hypoplastic No information regarding the family history was availabl except that th parents were not related

Case 3 S S This 5-day-old female had bilateral postaxial polydactyly of the hands and the feet She also had a butterfly vertebrae at Th6 and congenital heart disease the type of which is yet to be determined Th father was 26 and mother 24 years of age and th y were not related According to the parents there was no one in the family with congenital malformations

Case 4 M 1-day-old male had an extra digit at left He was th second child of this family The father was .6 and the mother 19 years of age Consanguinity between the parents and congenital malformations in the family were d nied On admission he was noted to have a tracheoesophageal fistula with oesophageal atresia in addition to the facial asymmetry and the absence of the right ear X ray examination showed fusion of the bodies of Th6–

Table 1 *General survey of the cases described*

Case	Sex	Type of polydactyly	Vertebral anomalies	Parents age Mother	Parents age Father	Consanguinity	Other anomalies
1	♂	Unilateral (Le[a])	0	23	24	—[b]	Omphalocele
2	♀	Unilateral (R)[c]	None	20	23	0[d]	Iliac hypoplasia (Le)
3	♀	Ulnar bilat Fibular bilat	Butterfly vertebrae Th_4	24	26	—	Cong heart disease (?)
4	♀	Radial (Le)	Fusion of the bodies of Th_8–Th_9, hemivertebrae Th_2–Th_6 and Th_9–L_1	19	26	—	Tracheo-esophageal fistule with esophageal atresia facial asymmetry absence of the right ear
5	♂	Radial bilat	Thoracic hemivertebrae	0	0	—	Facial asymmetry malformed ear (R) iliac hypoplasia (Le)
6	♀	Radial (R)	0	22	30	—	—
7	♀	Radial (R) Tibial Bilat	Extra thoracic vertebrae	0	0	+	Syndactyly (left hand and left foot)
8	♂	Triphalangeal thumbs bilat	Distal sacral agenesis	30	36	0	Right renal agenesis left undescended testes
9	♂	Radial (R)	None	19	24	—	—

[a] Le – Left [b] — – Absent [c] R – Right [d] 0 – Not recorded

Th9 and hemivertebrae involving Th2-Th6 and Th9-L1

Case 5 L K A 2 day old male was found to have extra thumbs bilaterally He also had facial asymmetry and a malformed ear X ray examination revealed left iliac hypoplasia and thoracic hemivertebrae The parents were not related and there was no history of congenital malformations in the family

Case 6 O M A 20-day old female had a supernumerary right thumb The parents were not related The father was 30 and mother 22 years of age Unfortunately no X rays of the spine were taken The parents denied the existence of similar or other congenital malformations

Case 7 U 2 day old male had an extra thumb at right associated with bilateral preaxial polydactyly of the feet In addition he had syndactyly between the 3rd and 4th left fingers and also between the extra digit and the first left toe Only a part of the spine was seen in his X ray films which showed an extra thoracic vertebrae No further information was available about the family except that the patient was the second child of this consanguinous family

Case 8 Y M S A 1½-month old male who had triphalangeal thumbs bilaterally The X rays of spine revealed distal sacral agenesis I V P revealed right renal agenesis He also had undescended testes at left No information with regard to the family history was available

Case 9 A A 3 day old male infant who had an extra thumb at right Skeletal X rays of the spine revealed no abnormalities He was the first child of unrelated young parents (father 24 mother 19 years of age) The family denied any history of congenital malformations on either side of the family

DISCUSSION

It is well known that congenital malformations may be multiple and that discovery of one should spur the search for others in the same patient Many such combinations of anomalies have been collected into recognized syndromes The purpose of establishing such syndromes is threefold Firstly, they stress that seemingly different malformations are correlated and should be considered as a unit Secondly, it stimulates a search for further components of the syndrome and finally it may lead to the discovery of the etiology through analysis of similar cases and hence perhaps may result in prevention when the pathogenesis is better appreciated

Many frequent combinations of malformations other than those known at present probably exist and remain unrecognized They may be infrequent enough to escape recognition or subtle enough to be missed on a cursory examination or of such a minor nature that little attention is focused on them The optimal procedure for delineation of a new syndrome is undoubtedly the one based on an etiology On the other hand when this is not possible one can collect a large number of patients with

Table 2 *Hand malformations (25 cases)*

	Preaxial	Postaxial	Unknown	Total
Polydactyly	12	2	3	17
Hypoplastic thumbs	3	—	—	3
Absence of the thumbs	3	—	—	3
Abnormality of the thumbs[a]	1	—	—	1
Triphalangeal thumbs	1	—	—	1
	20	2	3	25

2 further cases had polydactyly of the toes only
[a] Not defined

arefully investigated precisely documented nalformations and earch for recurring simi arities within this group However in the absence of such large prospective studies investi gation of sporadic cases or of their hospital records retrospectively will have to suffice al hough it should be remembered that a certain constellation of malformations may not neces sarily indicate ethiological uniformity Publica tion of such patients we believe may alert other investigators to look for additional cases and hopefully to publish them leading to a comprehensive delineation of the syndrome in time

This newly proposed syndrome of multiple malformations may actually constitute a good example for the potential importance of retrospective studies Since the publication of the first report on this subject several observations have appeared in the literature which helped greatly towards a b tter understanding of the proposed syndrome (1 3 9)

In Table 1 the malformations seen in our cases with imperforate anus have been pre sented Here the type of anorectal anomalies observed in individual cases have not been re corded As it is known imperforate anus is a d scriptive term and covers a heterogenous group of malformations varying from minor deformities to complex anorectal anomalies Among our patients there were 3 cases with anal stenosis (Ladd and Gross classification type I) (2) one with an anal membrane (type

Table 3 *Vertebral anomalies in 16 cases*

Hemivertebrae	7
Neural arch anomalies	7
Fused vertebrae	5
Increa ed number of vertebrae	5
Bifid vertebrae	3
Other anomalies	7

II) and another one with type IV deformity In the remaining 4 cases the anus was absent and the rectum was ending blindly (Type III) which is the commonest type of malformation en countered in clinical practice

The various malformations outside the ano rectal anomalies encountered among the 18 published cases with this entity together with tho e seen in our 9 patients are presented in Tables 2–4 Interestingly in 17 patients of a total of 25 (the remaining 2 patients had poly dactyly of the toes only) the hand malforma tion was in the form of polydactyly while 3 patients had ectrodactyly three had hypoplasia and one a triphalangeal thumb These indicate that contrary to the observations in mice as will be discussed later polydactyly is seen more often than other forms of hand malformations even if one removes the 10 cases reported in the initial communication since they were ascertained through their poly dactyly It should be recorded however that of the six cases reported by Tunte (9) none had an extra digit while among our cases as certained similarly through their anal mal formations 8 patients out of 9 had polydac tyly

Table 4 *Other malformations*

	No of cases
Tracheo-esophageal fistula	3
Agenesis of the kidney	2
Facial asymmetry	2
Malformed ears	2
Congenital heart disease	1
Agenesis of the lung	1
Hypospadias	1
Omphalocele	1
Agenesis of the spleen	1

There were 3 other cases with probable congenital heart disease

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is not affected In most of them the antero-ventral part of the left kidney is flattened and in the remaining it is hydropic The stomach is smaller than normal in many with a reduced caecum in some On the other hand in the Dh/Dh mice the stomach is much smaller and hydropic kidneys are usually seen in all There is no anus in 44° of them and similarly a gap in colon or rectum or a blind ending rectum i seen in the same percentage of these mice Reduction in the number of thoracic ribs and presacral vertebrae is seen in both Dh/ and Dh/Dh mutant mice although the malformations are mu h more severe among the latter (6)

The similarity between the findings observed in patients with this newly syndrome and in mice with dominant hemimelia is indeed striking. Certain differences however should be mentioned First of all the malformations involve generally the hind limbs in mice while the hand malformations seem to be more commonly encountered in man Secondly anal atresia is usually seen in the homozygot mutant mice who rarely survive more than a few days and in whom always oligodactyly rather than polydactyly is seen Finally dominant pattern of inheritance as in mutant mice has not been proven in man as yet In fact the role of hereditary factors in the etiology of this triad is uncertain So far with a possible exception of the family described by Fuhrmann (1) no evidence in this regard is available Fuhrmann described a family in which a 6-week old boy had an imperforate anus and his old r brother had the same anomaly in addition to unilateral radial polydactyly Interestingly the mother herself had a unilateral polydactyly (1) In our series however no similar malformations existed among the members of the seven families where detailed family history could be obtained

Similarly reports by others also failed to result in a positive family history It may be argued that some of these cases may be the result of a new mutation although all could not be considered as such It may be also speculated that the negative family history is the result of several factors such as low penetrance of presumed mutant gene decreased reproductive capacity and/or the short life span and hence the decreased fertility of the affected etc All these probably indicate that the possibility of a dominant heredity can not be excluded at present

The possibility of a chromosome disorder being the cause of this condition could not definitely be eliminated since chromosome studies have not been done in many of these cases However the available observations although admittedly limited give no indication as to the presence of a distinct chromosome aberration which might be responsible for this condition Environmental factors may also be responsible for this syndrome Although family histories failed to reveal any teratogenic agent this possibility can not at present be definitely ruled out All these indicate that further studies are in order to clarify this matter

The true incidence of this condition among the live born infants is yet to be established Among the 185 caucasian patients with polydactyly this combination of malformations was observed in 10 cases (5 4%) (5) However the incidence figure obtained from the above mentioned study due to the method of ascertainment used may only represent a large referral hospital population It is quite conceivable for instance that some of the patients with the milder form of polydactyly such as those with ulnar skin tags were treated at birth by their family physician and therefore would not be included in this particular study It should also be recorded that many of these cases with polydactyly (34°) had other major malformations which were the main reason for their hospitalization (5) All these indicate that the prevalance of this syndrome among the patients with polydactyly is in fact considerably below 5 Retrospective studies of the hospital records are expected to provide more reliable incidence figures for patients with imperforate anus since this latter condition will practically always necessitate hospitalization

One common finding to the reported cases seems to be the site of the hand malformation This was found to be preaxial in 20 instances out of 25 where it was recorded On two occasions the extra digits involved the feet only, but again the location was preaxial in one of them (Table 1)

Vertebral anomalies of various types and severity were present in 16 cases out of the total 21 patients in whom the X rays of the spine were available for examination In some patients the abnormality merely consisted of an increase in the number of the vertebrae while in others it was of more serious nature such as fusion of the vertebrae and hemivertebrae involving one or more vertebral bodies (Table 3) It is interesting that these deformities were presacral in 14 cases Although as stressed by Williams & Nixon (10) various forms of sacral deformities including sacral agenesis are not too rare in patients with imperforate anus usually few vertebral abnormalities are encountered above the sacral level in these patients

Malformations of the ribs were also relatively common among these patients They were found in 8 patients The types of malformations encountered again varied from patient to patient however hypoplasia and fusion of the costal bones as well as an increase in the number of them were among the more frequent abnormalities It may be that rib anomalies are more common in these patients than the observations so far indicate since at least in our series satisfactory X rays were not available in most of the cases However in some of the cases the malformations of the ribs were probably secondary to the vertebral deformities

Another interesting finding was hypoplasia of the ilium which was found twice in our series and again on three occasions in the initial report by Say & Gerald (5) These peculiarly involved the left side on all these occasions This predilection for one side has been noted in other deformities and its cause is still unknown

Among the remaining associated malformations tracheoesophageal fistula with esophageal atresia was seen on three occasions (Table 4) The diagnosis of congenital heart disease was made in 4 cases Although in the initial report on this subject no such case was encountered others subsequently published cases who had congenital heart disease in addition to the cardinal malformations In dominant hemimelia mutant mice this malformation does not usually occur However, in all these patients with the exception of the case reported by Tunte (9) the diagnosis of congenital heart disease was not definite and this possibility was raised because of the presence of a murmur It seems that more data are necessary before one reaches a final decision in this matter

Other infrequent malformations seen are also listed in Table 4 These include omphalocele facial asymmetry, malformations of the ears agenesis of the kidney and undescended testes among others Rectovaginal rectovesical and rectourethral fistulas were present in 6 cases This is an expected finding and should probably not be considered separately from the anal malformations

Dominant hemimelia mutants are a member of the luxoid group of mouse mutants This mutant was discovered in 1954 by Carter in Edinburgh (6) The extremity abnormalities in the heterozygous mice (Dh/) are confined to the preaxial site of the hind limbs The expression of it is quite variable since it is no more than a slight thickening and lengthening of the hallux in some offspring while in others it is in the form of a triphalangeal hallux or an extra digit on that side as well as syndactyly between certain digits In some others deficiencies are observed such as a loss of a digit or two or part of digit and even luxation and reduction in the length of one or both hind limbs In the Dh homozygotes (Dh/Dh) the hind legs are short and twisted and usually show oligodactyly The fore limbs reveal no abnormalities These mutant mice also have many visceral abnormalities Dh/ mice lack spleen while the rest of the lymphatic system

is not affected In most of them the antero-ventral part of the left kidney is flattened and in the remaining it is hydropic The stomach is smaller than normal in many with a reduced caecum in some On the other hand in the Dh/Dh mice the stomach is much smaller and hydropic kidneys are usually seen in all There is no anus in 44% of them and similarly a gap in colon or rectum or a blind ending rectum is seen in the same percentage of these mice Reduction in the number of thoracic ribs and presacral vertebrae is seen in both Dh/ and Dh/Dh mutant mice although the malformations are much more severe among the latter (6)

The similarity between the findings observed in patients with this newly syndrome and in mice with dominant hemimelia is indeed striking Certain differences however should be mentioned First of all the malformations involve generally the hind limbs in mice while the hand malformations seem to be more commonly encountered in man Secondly anal atresia is usually seen in the homozygot mutant mice who rarely survive more than a few days and in whom always oligodactyly rather than polydactyly is seen Finally dominant pattern of inheritance as in mutant mice has not been proven in man as yet In fact the role of hereditary factors in the etiology of this triad is uncertain So far with a possible exception of the family described by Fuhrmann (1) no evidence in this regard is available Fuhrmann described a family in which a 6 week old boy had an imperforate anus and his older brother had the same anomaly in addition to unilateral radial polydactyly Interestingly the mother herself had a unilateral polydactyly (1) In our series however no similar malformations existed among the members of the seven families where detailed family history could be obtained

Similarly reports by others also failed to result in a positive family history It may be argued that some of these cases may be the result of a new mutation although all could not be considered as such It may be also speculated that the negative family history is the result of several factors such as low penetrance of presumed mutant gene decreased reproductive capacity and/or the short life span and hence the decreased fertility of the affected etc All these probably indicate that the possibility of a dominant heredity can not be excluded at present

The possibility of a chromosome disorder being the cause of this condition could not definitely be eliminated since chromosome studies have not been done in many of these cases However the available observations although admittedly limited give no indication as to the presence of a distinct chromosome aberration which might be responsible for this condition Environmental factors may also be responsible for this syndrome Although family histories failed to reveal any teratogenic agent this possibility can not at present be definitely ruled out All these indicate that further studies are in order to clarify this matter

The true incidence of this condition among the live born infants is yet to be established Among the 185 caucasian patients with polydactyly this combination of malformations was observed in 10 cases (5 4%) (5) However the incidence figure obtained from the above mentioned study due to the method of ascertainment used may only represent a large referral hospital population It is quite conceivable for instance that some of the patients with the milder form of polydactyly such as those with ulnar skin tags were treated at birth by their family physician and therefore would not be included in this particular study It should also be recorded that many of these cases with polydactyly (34) had other major malformations which were the main reason for their hospitalization (5) All these indicate that the prevalance of this syndrome among the patients with polydactyly is in fact considerably below 5% Retrospective studies of the hospital records are expected to provide more reliable incidence figures for patients with imperforate anus since this latter condition will practically always necessitate hospitalization

At this institution nine cases, presumably representing this syndrome were encountered among 258 cases with imperforate anus (3 4%) It is interesting that a similar figure was found in other series Tunte (9) reported 6 patients with hand malformations among 103 imperforate anus cases and in a report by Stevenson et al (7) on the occurrence and type of congenital malformations encountered in 24 centers in 16 countries we could find 5 possible examples of this syndrome among 110 cases with imperforate anus (4 5%) From these figures an estimate of the incidence of this condition among the live born infants can be made Since the incidence of imperforate anus is variously reported to be one in 1 000 to 5 000 caucasian live births (roughly 1/3 000) and since this syndrome is encountered in about 3 to 5% of the cases with imperforate anus, then the incidence of this condition among the caucasian liveborn infants should be one on 20 000 to 150 000 (1/75 000)

Finally it should be mentioned that in this communication patients having imperforate anus and hand malformations with or without vertebral anomalies have been considered There were 5 patients in whom no abnormalities related to the spine could be found The reason for their inclusion is the fact that in many well established syndromes some of the cardinal features may be missing It is hoped that the publication of further new observations will bring more light to the understanding of this syndrome of dysmorphogenesis

SUMMARY

Nine patients having imperforate anus and hand malformations with or without vertebral anomalies have been presented The similarity of the findings in these patients to those seen among the members of the luxoid group of mouse mutants such as the dominant hemimelia (Dh) mutants has been emphasized Similar cases reported in the literature have been briefly reviewed

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PURPURA AND ACETYLSALICYLIC ACID THERAPY

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Th o currence of purpura in a child often faces the paediatrician with the problem of diagnosing a disturbance with an ill defined etiology or a disease with bad progno is For tunately this is not always the case and it was a relieving experience to find that in three of our patients with purpura this symptom was most probably caused by the ingestion of a normal dose of acetylsalicylic acid

Purpura as a complication of acetylsalicylic acid therapy is not unexpected in view of the findings of Evans et al (4) Weiss et al (20) and O Brien (11 12) who independently of each other observed in 1968 that acetylsali cylic acid affected the "in vivo and "in vitro aggregation of blood platelets mainly by an inhibition of the release of adenosine 5 -di phosphate (ADP)

Our case reports are as far as we know the first clinical descriptions of a purpura due to this mechanism

CASE REPORTS

Case 1

A 10-month-old female infant was referred to the hospital because of purpura She i the first child of healthy and unrelated parents There is no history of familial haemorrhagi d sease The baby was born weeks before term birthweight was 2 700 g Physi cal and psychomotor d velopment were normal She always received a properly balanced diet Two days before admiss on she developed a mild cough for which she was given on three consecutive nights one suppository containing 65 mg of acetylsalicylic acid On the morning of admission sh developed purpuric spots on her face The physical examination on ad mission revealed a normally developed child with a weight of 8 750 g (P25) and a height of 70 cm (P25) The temperature on admission was 38 6C the heart rate 150/min and the blood pressure 90/50 mmHg She di ln t look very ill and was not pale at all but she presented numerous petechiae These were pin point sized multiple and very densely spread over the face and ne k but scarcely on the arms and trunk There was neither bruising nor erythematous rash nor urticarial wheals The Rumpel Leede test was positive The further physical findings were nor mal except for a purulent rhinopharyngitis Liver and spleen were not palpable A treatment with am picilline and nosedrops was started Routine blood and urine analyses gave normal values The platelet count was 556 000/mm Nose and throat culture yielded no pathogens Five days after admission all petechiae had disappeared and since then th baby remains well

Case 2 and 3

A mother presented at the out patients department with her two children both girls respectively 9 years and 14 months old because of purpuric spots

We were told that the younger child presented this phenomenon for the second time The moth r made the remark that on both occasions the baby was teething and for this reason was given acetylsalicylic acid as an analgesic The girl of 9 had received this drug because of some fever Both children received approximately 30 mg/kg/day for 4 days Their parents are healthy and unrelated they have three children all of them in good health There is no family history of bleeding tendency but it is note worthy that the father on two occasions with an interval of 3 years had a severe gastric bleeding

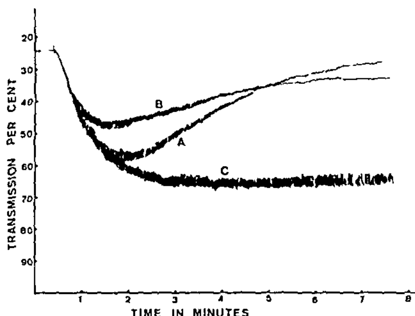


Fig 1 Platelet aggregation by ADP A case 1 (first observation) B case 1 (second observation) C control

after a single intake of respectively 2 g and 1 g acetylsalicylic acid

On clinical examination both children looked healthy Physical and psychomotor development were normal The younger child had multiple petechiae roundshaped with a diameter of half to one mm in the neck and at the upper trunk The older girl only had a few identical petechiae about ten spread over the anterior part of the neck

The rest of the clinical findings were all normal Routine blood and urine analysis also gave normal values The platelet count was 230 000/mm

After exclusion of the most obvious causes it was assumed that in these three patients the purpura might be due to a defect in the platelet aggregation following acetylsalicylic acid therapy Unfortunately only two of the three patients were available for further study of the platelet function

MATERIAL AND METHODS

The blood for the haemostasis tests and for study of the platelet function was obtained by venous puncture and collected in plastic tubes containing 1 part of a solution containing 3 13 % sodium citrate for 9 parts of blood Platelet rich plasma (PRP) was prepared by centrifuging blood at 400 g for 10 min Platelet poor plasma (PPP) was obtained by centrifuging platelet rich plasma at 6 000 g for 10 min These procedures were performed at room temperature Adenosine 5 diphosphate (ADP) and adrenaline were supplied by Stago Solutions containing 20 μg/ml were prepared just before use A preparation of phospholipids Thrombofax (Ortho) was used without further dilution Russell s viper venom (Stypven Burroughs Wellcome) was used as a 1/100 000 dilution in isotonic saline Calcium was added from a 0 05 M solution of $CaCl_2$

The platelet counts were performed using blood diluting pipettes (Unopette Becton Dickinson) Platelet aggregation in PRP was followed with Borns photometric technique (1) using an E E L 401 Absorptiometer (Evans Electroselenium Ltd) with built in stirrer and water jacket for temperature control The absorptiometer was connected to a pen recorder (Vitatron) for automatic registration of variations in transmitted light All experiments were performed at 37 C PRP was adjusted to 300 000 platelets/mm³ by diluting with PPP of the same subject For the aggregation tests 0 2 ml of aggregating agent was added to 0 8 ml of PRP

Prothrombin (factor II) and factor V were assayed according to Stormorken (17) The one stage prothrombin time was performed according to Quick (15) and the P and P test according to Owren & Aas (14) The plasma fibrinogen level in mg% was determined using the Fibrin Polymerization Time (FPT) test (18) and the thrombin time was measured as described by Vermylen & Verstraete (19)

Clot retraction was evaluated visually in whole blood after 1 hour incubation at 37 C and the result was expressed arbitrarily as a value between 0 (absence of retraction) and 4 +

Partial thromboplastin time was determined according to Langdell et al (9) and the availability of platelet factor 3 (PF 3) according to Spaet & Cintron (16)

RESULTS

Case 1

The aggregation of the platelets in the patient PRP was studied for the first time 5 days after interruption of the acetylsalicylic acid therapy The platelet aggregation by adding ADP was normal but was followed by a rapid disaggregation (Fig 1) The effect of adrenaline on the platelet aggregation is shown in Fig 2

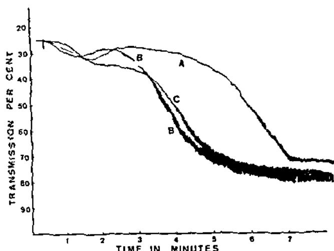


Fig 2 Platelet aggregation by adrenaline A case 1 (first observation) B case 1 (second observation) C control

Usually this aggregation occurs in two phases a first one which depends on adrenaline itself and a second one which depends on release of endogenous ADP In our patient the first wave of aggregation was normal but the second one appeared with an appreciable delay

As was shown by the Gaetano et al (2) "Thrombofax which contains lipid known as cephalins induces a strong aggregation after a latent period of 60–80 sec This action of Thrombofax seems to be similar to that of collagen The addition of thrombofax to PRP of our first patient induced aggregation only after a prolonged period of time (Fig 3) The availability of PF 3 was normal

After 20 days during which period the patient did not receive acetylsalicylic acid all tests were repeated As shown in Figs 1 2 and 3 the alterations of the platelet function which were observed at the first investigation had completely disappeared

The other haemostasis tests performed in this patient during and after acetylsalicylic acid therapy gave normal values with exception for the platelet counts which were rather high (Table 1)

Case 2

The aggregation tests performed on the PRP of this patient (the 9 years old girl) during

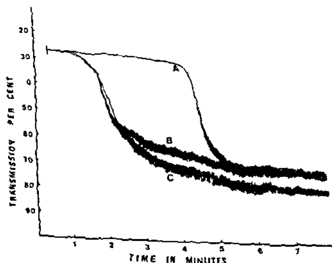


Fig 3 Platelet aggregation by phospholipids A case 1 (first observation) B case 1 (second observation) C control

Table 1 *Results obtained for the various hemostasis tests performed in case 1 and 2 while under the influence of acetylsalicylic acid (A), and after interruption of the drug for more than 15 days (B)*

	Normal values	Case 1		Case 2	
		A	B	A	B
Blood platelets/mm³	140 000/300 000	556 000	500 000	230 000	260 000
Bleeding time	30 –/3	4 50	3	5 20	2 10
Clot retraction	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +
Prothrombin time					
(undiluted plasma Quick)	100	100	100	100	100
(diluted plasma Owren)	100	100	80	100	75
Factor V	100	100	100	100	95
Prothrombin in plasma	100	100	100	100	95
Thrombin time	18–24 sec	18 sec	20 4 sec	18 5 sec	21 5 sec
Fibrinogen (total blood)	90–210 mg	220 mg	222 mg	147 mg	95 mg
Partial thromboplastin time					
(without activation)	100 sec	76 sec	92 sec	87 sec	65 sec
(with activation)	< 60 sec	40 sec	47 sec	28 sec	34 sec

acetylsalicylic acid therapy showed similar alterations as in case 1 However, the disturbances of the platelet function were more pronounced Addition of ADP caused a normal aggregation, but a more rapid disaggregation ensued The second wave of aggregation after addition of adrenaline was completely absent and the clumping induced by Thrombofax was markedly reduced All these alterations of the platelet function had completely disappeared at a second investigation three weeks after the interruption of the acetylsalicylic acid therapy

As in case 1 the availability of PF 3 and the other hemostasis tests were normal (Table 1)

DISCUSSION

Purpura in a patient with normal platelet count can be due to a vascular defect usually described as vasculitis or to an abnormality of the platelet function

Acetylsalicylic acid in a toxic dose has been reported to be responsible for purpura Indeed petechial haemorrhages can be a prominent feature at post mortem examination of individuals dying from acetylsalicylic acid poisoning (7) Non thrombocytopenic purpura has also been observed in patients receiving a normal dose of acetylsalicylic acid but this disturbance was believed to be due to hypersensitivity (6)

In our patients overdosage as well as idiosyncrasy can be excluded A single test dose of acetylsalicylic acid (5 mg/kg) given to our patients did not cause a reappearance of the symptoms

Frick (5) reporting in 1956 on haemorrhagic diathesis caused by salicylate therapy stated that acetylsalicylic acid induced a positive Rumpel Leede test and an abnormal bleeding time in his patients To explain this phenomenon at that time he forwarded the hypothesis of an increased capillary fragility However the recent investigations of the effect of acetylsalicylic acid on blood platelets suggest that also in these patients a disturbance of the platelet function rather than a vascular defect might be responsible for his findings

The normalization of platelet function 20 days after interruption of the acetylsalicylic acid therapy provides evidence that the abnormal behaviour of platelets in our patients was due to the intake of acetylsalicylic acid and cannot be considered as a Portsmouth syndrome (10)

This effect of acetylsalicylic acid on platelet function was first observed in 1968 independently by Evans et al (4) Weiss et al (20)

and O Brien (11 12) and has been studied intensively during the last 2 years Ingestion of acetylsalicylic acid causes an impaired aggregation of platelets mainly by inhibiting the release of endogenous ADP

The "in vivo" release of ADP from stimulated platelets contributes to the adhesion of more platelets to those already stuck to a site injury Acetylsalicylic acid by interfering ith this release inhibits this phase of aggrega on and may in this way affect the hemostatic roperties of platelets Therefore it is not unexpected that it can induce purpura in a patent especially if an additional local factor rovokes some capillary damage The dense ppearance of purpuric spots on the face and eck of our first patient might be due to the nteraction of the disturbed platelet function nd a local increase of pressure in the capillaries e i during coughing A similar problem an be found in the haemorrhagic effect of acetylsalicylic acid in the gastro intestinal ract Such bleeding was first attributed to local gastro-intestinal irritation (3) but its occurrence after intravenous administration (8) made Weiss (20) to suggest that this haemorrhagic action is partly due to impaired release of platelet ADP

Noteworthy is the fact that the father of our patients described under case 2 and 3 suffered from a severe gastric bleeding on two occasions after a single intake of respectively 2 g and 1 g of acetylsalicylic acid Two of his children developed purpura while taking the same drug These phenomena can be independent of each other but they could as well be due to some familial predisposition to develop this impaired platelet function after ingestion of acetylsalicylic acid The question arises whether this predisposition might not explain the rather low frequency of these clinical complications

The alterations of platelet function after a single ingestion of acetylsalicylic acid remain detectable four to seven days after the intake although the serum salicylate level is raised only for a few hours after ingestion of the drug This fact suggests that platelets and presumably megakaryocytes are specifically and irreversibly modified by acetylsalicylic acid and that only after 4 to 7 days sufficient normal platelets reappear in the circulating blood thus normalizing the platelet aggregation tests (12)

It is also important to stress that many of the compounds sharing one or more of the pharmacological properties of acetylsalicylic acid exert an inhibitory effect on the platelet aggregation (13) The following drugs were found to have this inhibitory action "in vitro meclofenamic acid acetylsalicylic acid, indomethacin (Indocid) ibuprofen mefanamic acid (Ponstan) amidopyrine (Pyramidon) destro-propoxyphene hydrochloride (Doloxene) paracetamol ibufenac (Dytransin) and phenylbutazone (Butazolidin) In contrast oxyphenbutazone (Tanderil) phenacetin gentisic acid codeine dihydrocodeine and sodium salicylate did not have this inhibitory effect

From our clinical observations and these studies we would like to draw two conclusions Firstly that the possibility of a platelet dysfunction due to an inhibition of ADP release following acetylsalicylic acid therapy should always be considered in the differential diagnosis of a non thrombocytopenic purpura Secondly it would be safer not to use acetylsalicylic acid and the other anti inflammatory compounds mentioned above in children with bleeding tendency as for instance in the thrombopenic the leucemic and the hemophilic child

SUMMARY

Three cases are reported of children presenting purpura while taking a normal dose of acetylsalicylic acid since a few days Studies of the platelet function showed an impaired platelet aggregation by an inhibition of ADP release It is concluded that this mechanism is responsible for the clinical findings and that an acetylsalicylic acid therapy should always be considered in the differential diagnosis of a non thrombocytopenic purpura

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Purpura in a patient with normal platelet count can be due to a vascular defect usually described as vasculitis or to an abnormality of the platelet function

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CHRONIC REGIONAL ENTEROCOLITIS (MB CROHN) IN CHILDREN AND ADOLESCENTS

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During the last decade a marked increase in the occurrence of Regional Enterocolitis in adults has been observed in Sweden (10) In our institution a corresponding increase was noted in the pediatric age group (including children and adolescents under 16 years) Prior to 1959 isolated cases only were seen while a regular annual occurrence of 2–5 cases has been noted during the last decade This devel opment is similar to that reported previously with reference to Ulcerative Colitis in children (11) So far little attention has been paid to the specific pattern of the disease in children (1 6 8 15 18 21) A report of our experi ences in diagnosis and treatment of a 10-year series therefore seems timely

MATERIAL

36 patients with Regional Enterocolitis were treated from 1959 to and inclusive of 1969 24 boys and 12 girls The majority 31 have been subjected to surgical therapy The age distribution at onset and at opera tion is shown in Table 1 Most patients were above 9 years of age at onset The diagnosis has been con firmed by microscopy of the resected specimens showing the typical picture of granulomatous entero colitis In the 5 cases not operated upon the diagnosis was based on typical clinical and radiological find ings

CLINICAL FINDINGS

Symptoms and signs In our series an insidious onset was very common and was often mis interpreted in 13 patients more than 2 years elapsed from the appearance of the first symp toms to the establishment of the diagnosis in 7 patients the disease remained undiagnosed for more than 4 years Non specific symptoms fatigue anorrhexia weight loss and retardation of growth were of common occurrence In some patients retardation of puberty was evident (Fig 1) General symptoms sometimes pre ceded the local manifestations for a long period of time The most common local symptoms were abdominal pains (82%) and diarrhoea (88%) Diarrhoea was as a rule less severe than in Ulcerative Colitis Only one third of the pa tients had bloody stools 9 patients had perianal fistula and/or abscess formation 3 of them as a presenting symptom A palpable mass in the abdomen was encountered only in 4 cases Intraabdominal fistulation occurred in 2 pa tients in 1 case to the bladder 7 patients had extraintestinal manifestations (arthritis erythema nodosum or liver damage)

The following case report illustrates a common type of clinical course Boy born 1950 excema in infancy 1957 prolonged fatigue and poor appetite after family infection with influenza Anemia and high sedimenta tion rate found at repeat examinations by school physicians and in out patient clinics no diagnosis Intermittent diarrhoea and abdominal pain since 1961 Barium enema and intestinal series 1961 showed typical picture of Crohn s disease of terminal ileum and proximal colon (Fig 2) 1961–1964 intermittent severe gonitis treated with exsufflation and local in jections of prednisolon Patient also treated with salicylazosulphapyridine high caloric protein rich diet vitamine and (for shorter periods) with prednisolone

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Table 2 *Localization*

Localization	No of patients
Small intestine only	4
Colon only	2
Small intestine and colon	20
Colon and rectum	1
Small intestine and colon and rectum	4
Total	31

the terminal ileum with irregularly contoured mucosa and stenosed lumen The *localization* of the disease is shown in Table 2 27 of the 31 patients had changes in the colon most of them with the small intestine engaged as well Skip areas cobble stone pattern and pronounced strictures were frequent findings The earliest sign of colon changes was segmentally increased haustration Later on dehaustration and often transverse mucosal ulcerations could be seen

Table 3 *Common and distinctive features in ulcerative colitis and regional enterocolitis*

	Ulcerative colitis	Regional enterocolitis
Localisation		
Small intestine	– (except backwash ileitis)	+ + –
Colon	– +	+ +
Rectum	+ +	+
Skip areas	–	+ +
Pathology		
Strictures	+	+ + +
Abscess & fistula formation	–	– + +
Perforation diffus peritonitis		–
Cancer	+ +	–
Granulomatous inflammation	–	+ + +
Giant cells	–	+ +
History		
Marked anorrhexia	–	+ +
Abdominal pains	–	+ +
Blood in stools	+ + +	+
Extraintestinal manifestations	+ –	+
Retardation	+ +	+ + +
Remissions	+ +	–
Laboratory findings		
Sideropenic anemia	+ + +	– + +
B_{12}-deficiency	–	
Protein leakage	+ +	+ +

Table 4 *Procedures performed at primary surgery*

Procedure	No of patients
Ileocoecal resection	10
Resect of dist ileum and/or total colectomy + e a	8
Resect of dist ileum and hemi colectomy + e a	5
Resect of dist ileum + pancoloproctectomy + ileostomy	4
Separate resect of small and large intestine	3
Resect of dist ileum and colectomy + ileostomy	1
Total	31

Some of the radiological features are demonstrated in Fig 2

Eklof & Gierup (7) studied the retrorectal soft tissue space in 160 normal children and in 28 children with Regional Enterocolitis in 8 of whom the space was increased The increase was generally less marked than in Ulcerative Colitis and did not aid materially in the differential diagnosis At operation these cases showed a marked proctitis–periproctitis

DIAGNOSIS

Preoperative diagnosis was based on clinical and radiological findings In the early stages absence of local symptoms and signs were a common cause of diagnostic difficulties in particular in differentiating Regional Enterocolitis from Malabsorption Collagene Disease and Psychological Disturbances Later on the differentiation of local symptoms from those in Ulcerative Colitis was the main diagnostic problem A synopsis of similarities and differences between the two diseases has been compiled in Table 3 In regional Enterocolitis abdominal pain and pronounced anorrhexia are often encountered in Ulcerative Colitis these symptoms are rare Diarrhoea is usually less marked in Regional Enterocolitis and presence of blood less common Localisation to the small intestine and the radiological characteristics listed above are as a rule decisive in distinguishing between the two diseases Peri

Table I *Age at onset and at operation in regional enterocolitis*

Age (years)	At onset	At operation
3–5	1	
6–8	4	
9–12	21	8
13–16	5	21
17–19		2
Total	31	31

Results of therapy unsatisfactory patient invalidized by arthritis moderate growth retardation

1965 operated with resection of diseased bowel The specimen showed changes typical of granulomatous enterocolitis

Uncomplicated post operative course quick restoration of appetite and strength weight gain 9 kg in 6 months normal pubertal development regression of joint symptoms hemoglobin and sedimentation rate normalized Normal school achievement later trained as clerk 1969 deterioration of general state of health poor appetite weight loss slight joint symptoms sedimentation rate moderately elevated X ray examination demonstrates relapse in colon re operation planned

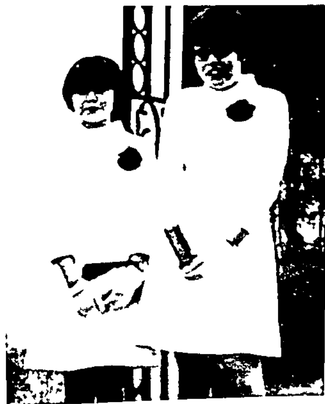


Fig 1 Growth retardation in chronic regional enterocolitis Patient G S (left) 13 years old symptoms appr 5 years Control identical twin B S (right) healthy



Fig 2 Typical X ray picture in regional enterocolitis in terminal ileum coecum and ascending colon (courtesy by Dr U Rudhe Karolinska Sjukhuset)

LABORATORY FINDINGS

An increased sedimentation rate was found in all patients occasionally extreme values (> 100 mm/1 hour) were noted The majority of patients showed a sideropenic anemia and an increased number of blood platelets (> 400 000/ mm³) About half of the patients showed hypoproteinemia hypoalbuminemia or hypergammaglobulinemia Intestinal protein leakage was demonstrated in 2 patients investigated in this repect

RADIOLOGY

Barium enema and barium meal studies were performed in all patients as a rule repeatedly The results in the first 15 cases have been reported previously (19) The most common finding was thickening of the intestinal wall in

few exceptions this occurred within the first 2 years postoperatively

An assessment of the radicality of the operation based upon re examination of the X ray films operative findings and microscopical findings of the resected specimen was performed (Table 8) In half of the cases (15) only the operation was judged to be radical In 12 cases radicality was questionable and in four patients resection was incomplete All of the patients in the last group had a recurrence In the radically resected and in the questionable groups the recurrence rate was about 40

8 recurrences out of 11 in the radically resected and questionable groups occurred in patients with wide spread disease 4 out of 5 cases with ileorectal anastomosis had a recurrence Three of these patients showed a thickening of the retror ctal tissue space proctitis or perianal fistula prior to operation

Among the 15 patients with recurrence after primary surgery 9 have so far been subjected to re-operation In one the result was good in four there was a new recurrence in four the follow up time is less than 2 years Two patients were operated upon a third time in both there was again a recurrence

DISCUSSION AND CONCLUSIONS

The increased incidence of Regional Enterocolitis in recent years seems in part to be explained by an increased awareness and more adequate diagnostic measures As in Ulcerative Colitis however a true increase seems highly probable The diagnostic difficulties encountered in the early stages of the disease presenting with nonspecific symptoms are evident Weight loss retardation of growth and puberty and increased sedimentation rate in patients with fatigue and anorrhexia should arouse suspicion of a chronic disease including the possibility of a diagnosis of Regional Enterocolitis Barium meal and barium enema studies should be included in the routine examinations of such patients In particular the importance of gastrointestinal X ray studies in patients with chronic citating or recurrent perianal fistulas should be stressed

Weight and sedimentation rate are good indicators of the activity of the disease abnormal finding postoperatively are indicative of a recurrence (23)

In recent years an increasing number of cases with colonic localization has been reported (2 4 13 22) In large series the incidence of colonic disease is reported as varying between 10–50% (5 10 17) In our series 27 out of 31 cases treated surgically had changes in the colon A high incidence of colon involvement thus would seem to be typical for the disease in children and adolescents Mosely et al (15) however found colonic changes in 4 patients only out of a total of 28

Extraintestinal manifestations seem to be more common in Ulcerative Colitis than in Regional Enteritis A comparison between our material gives the figures as 50 and 20 respectively

Restraint with surgery has formerly been recommended in view of a postulated tendency to spontaneous remission and of the risque of postoperative recurrence (12 17) In our series the results of conservative treatment were disappointing and did not prevent progression of the disease Similar results were reported by Anfanger (1) Cornes & Stetchet (4) and Jones & Lennard Jones (9) We therefore felt justified in trying a more active surgical approach As regards technique most surgeons nowadays consider a one stage radical resection of all diseased bowel to be the method of choice

Recurrence rates following primary surgery of between 20 and 65% have been reported (3 8 14 17 23) It has been claimed that the recurrence rate is lower when the disease is located in the colon (4 13 20) In our own series with its high incidence of colonic localization the recurrence rate was about 50 indicating that colonic localization does not seem to offer protection from a high recurrence rate

The importance of radicality is indicated by the fact that all of the 4 patients in our series

Table 5 *Length of follow up periods after primary surgery*

Time years	No of patients
<1	5
1–2	5
2–4	11
>4	10
Total	31

anal abscess or fistula formation is strongly indicative of Regional Enterocolitis Microscopical examination is however, sometimes necessary for the differentiation especially when the lesion is confined to the large bowel

THERAPY

In the early part of this series prolonged intensive conservative treatment was favoured administration of protein iron salicylazosulphapyridine blood transfusions vitamines and corticosteroids The *tendency to remission* however, proved to be slight *With corticosteroids* temporary improvement could be *achieved but* in general progression of the disease soon occurred The adverse effects of the disease on weight, growth and puberty prolonged disability and inefficacy of conservative treatment encouraged us to a more active approach *Chronic* progressive disease was the main indication for surgical treatment (23 patients) Severe arthritis was the indication in 2 cases 6 emergency operations were performed three because of intestinal obstruction two due to an acute fulminating type of *disease and one* on the assumption of appendicitis The time elapsing between the first appearance of symptoms and surgery has been rather short—as an average 3 years (In our material of Ulcerative Colitis the corresponding period was around 7 years)

The principle of surgical treatment adopted has been resection of all diseased bowel whe possible with restoration of intestinal continuity The various surgical procedures used are listed in Table 4

The length of follow up periods after primary surgery is shown in Table 5

Table 6 *Postoperative complications*

Type	No of patients
Intestinal obstruction	6
Leakage from anastomosis	2
Disruption of wound	2
Convulsions	1
Total	*11*

Table 7 *Results of primary surgery*

	Follow up >2 years	Follow-up <2 years	Total
No recurrence	8	8	16
Recurrence	13	2	15
Total	21	10	31

Table 8 *Assessment of radicality*

	Radical	Non radical	Questionable	Total
	15	4	12	31
Recurrence	6	4	5	15

EARLY RESULTS

Neither operative nor late mortality has been encountered The immediate results of surgery were good All patients were relieved of pains the diarrhoea diminished appetite and mood improved The average weight gain during the fist 6 months postoperatively was 6 kilograms *Haemoglobin* and sedimentation rate values normalized *Patients with ileostomy* were well adapted and have recommended the operation to other patients

Early postoperative complications causing temporary troubles only are listed in Table 6 Only two out of nine patients on steroid medication had such complications

LATE RESULTS

Late results of primary surgery are shown in Table 7 15 patients had a recurrence With

appearances in granulomatous colitis *Am J Roentgenol 108* 674 1970
8 Van Heerden J A Sigler R M & Lynn H B Regional enteritis in children *Mayo Clin Proc 42* 100 1967
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20 Shofield P F Some aspects of Crohn s disease *Dis Colon & Rectum 10* 262 1967
21 Silverman F N Regional ileitis in children *Aust Paediat J 2* 207 1966
22 Snellman B & Westerholm P Crohn s disease of the colon and rectum *Dis Colon & Rectum 9* 427 1966
23 Wenckert A Brahme F & Nilsson T Operationsindikationer vid Crohns sjukdom *Nord Med 83* 334 1970
24 Winkelman E I Regional enteritis in adolescence *Ped Clin N Amer 14* 141 1967

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who in retrospect proved to have had non-radical resections performed developed recurrences (or, rather, continued disease) Similar findings have recently been reported by Wenckert et al (23)

A recurrence rate of 40% was, however, found even when radical resection has been performed In our experience the extent of the lesion is an important prognostic factor in particular the high risk of recurrences after extensive resections of small bowel and/or colon with ileo rectal anastomosis should be stressed (13) In the case of extensive disease with rectal involvement leaving the rectum behind seems to be less favourable in Regional Enterocolitis than in Ulcerative Colitis Before such operations are performed a careful investigation of retrorectal soft tissues, rectal mucosa and peri anal manifestations should be performed

In view of the smooth adaptation of children to ileostomy we have not hesitated to perform a pancoloproctectomy when indicated by the extension of the disease So far we have not seen malabsorption develop postoperatively In cases where extensive resections of the distal ileum has been performed vitamin B12 should be given profylactically

In order to decrease present high recurrence rates it has been suggested (10 23) that resections should be more radical including segments of radiologically and macroscopically normal bowel in particular in colon resections Postoperative treatment with salicylazosulphapyridine and/or corticosteroids has also been suggested as a possible prophylaxis against recurrences None of these methods have however had an adequate trial so far

An interesting approach has been reported by Oberhelman et al (16) achieving promising short term results by a temporary diverting ileostomy—a procedure which has been tried and abandoned in the treatment of Ulcerative Colitis

The high incidence of recurrence makes surgery less encouraging in Regional Enterocolitis than in Ulcerative Colitis On the other hand the progressive nature of the disease and the poor effect of conservative treatment force us to continue the surgical defense until research has given us new weapons against this puzzling disease

SUMMARY

A series of 36 children and adolescents with chronic regional enterocolitis is presented An apparent increase in the occurrence of the disease is noted Clinical and roentgenological findings are reported A high incidence of colonic localization was found and is probably typical of a paediatric series There were difficulties in establishing an early diagnosis mainly due to the common occurrence of non specific symptoms in the early stages of the disease The guiding principles for the differentiation between regional enterocolitis and ulcerative colitis are discussed The results of conservative treatment were poor The majority of patients (31) were subjected to surgical therapy, the main indication being chronic progressive disease The immediate results of surgery were good In respect of late results a recurrence rate of about 50% was encountered Extensive disease especially when treated with resection and ileo rectal anastomosis seemed to have an unfavourable prognosis The importance of radical resections is stressed

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EPIDIDYMITIS IN CHILDREN

A Brief Review together with Reports of Six Cases

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The occurrence of acute epididymitis during the prepuberal period is considered a rare phenomenon In patients in that age group, an acutely developing lesion of the scrotum will usually be torsion of the testis or incarcerated hernia Certainly other diseases may also be responsible but as a rule they will not give rise to differential diagnostic doubts (1 6 13)

Epididymitis in children is not mentioned in the current text books on paediatry and data are scanty in the literature on the matter (2 6 13) Within the recent 4 year period we have seen 6 cases in the paediatric department in Glostrup Hospital, in two of these cases the development formed part of Schonlein Henoch s purpura This complication has been mentioned only twice before (7 9) and publications have never included pictures of biopsies from epididymis which at microscopic examination presented the characteristic changes known from Schonlein Henoch s purpura and other systemic diseases

CASE REPORTS

Case 1

A 6 year old boy was hospitalized on the suspicion of an acute abdomen Hitherto he had been healthy and in particular he had no allergic symptoms and was not either predisposed to allergy On the day of admission pains reminding of abdominal colics set in At the physical examination the abdomen felt soft to the touch although there was some diffuse tenderness In the course of the first few days the condition aggravated and the boy discharged sanguinolent faeces microscopic examination revealed haematuria and the temperature was fluctuating He went into shock and the condition did not improve until treatment by parenterally applied fluid was instituted Five days after admission cutaneous haemorrhages typical of Schonlein Henoch s purpura appeared, but neither oedema nor articular swelling occurred Three day later reddening swelling and tenderness of the scrotum set in together with turgidity of the scrotal content and swelling of the right funicle On the suspicion of torsion of the testis an operation was performed at which marked oedema and swelling of the epididymis was found in particular at the site of the caput while the testis was of normal appearance A biopsy taken from the caput revealed an inflammatory infiltration with many eosinophil cells and necrosis of the vascular wall whereas the ductules were well preserved Symptoms disappeared within a few days and nothing except a moderate proteinuria and microscopically visible haematuria persisted at the time of discharge

Four months later the boy was re admitted on account of abdominal pain and crural petechia Four days after admission he had an episode of right sided clonic spasms combined with corresponding left sided EEG changes Spasms had not previously been experienced and the episode should probably be taken as an influence on the brain involved in Schonlein Henoch s purpura just like the persistent glomerulonephritis It was not until 9 months after the first hospitalization that he became completely symptom free and that all laboratory tests including tests of urine gave normal findings

Laboratory tests SR 26 Leucocyte count 6 300/mm and normal distribution Thrombocyte count 500 000–1 000 000 mm throughout the first 4 months No eosinophilia AST 400 (elevated) normalized after 2 months ASH normal findings Culture of urine no growth and no leucocyturia Parotitis complement fixation test negative GO and GP transaminase lactic acid dehydrogenase serum electrophoresis blood urea and plasma creatinine normal findings Emmi Weil and Widal tests negative Coagulation time

bleeding time and prothrombin values normal Swabs from the throat showed growth of β haemolytic streptococci Pathogenic intestinal bacteria were not in evidence nor were coxsackie virus echo- and poliomyelitis vira demonstrable in faeces *Radiography of* thorax and intravenous urography normal findings BP normal ECG normal findings

Case 2

A hitherto healthy boy of less than 6 years was hospitalized on the suspicion of an incarcerated inguinal hernia There was no history of allergy A few hours before admission he felt pain in the scrotum At the physical examination the right half part of the scrotum was found to be reddish and swollen the scrotal content had increased and the funicle had thickened At operation the epididymis was found to be swollen but the testis was normal A biopsy taken from cauda epididymis revealed changes similar to those observed in case 1 except that they were le s marked Ten days after admission a haemorrhagic exanthema typical of Schonlein He noch s purpura appeared on both legs Oedema of the ankle was negligible The patient had no medication during hospitalization and his temperature remained normal

Laboratory tests SR 17 AST and ASH normal findings Thrombocyte count 700–850 000 mm Microscopy of urine normal findings Culture of urine no growth Parotitis complement fixation test negative Intravenous urography revealed splitting of the left-sided renal pelvis otherwise normal findin s Cysto-urethrography of micturition normal findings

Case 3

A boy aged 3 months was hospitalized on the suspicion of an incarcerat d inguinal hernia Pregnancy and parturition had been normal Weight at birth 4 200 g No history of allergic diseases Since his birth there had been a tendency to regurgitation which aggravated when he at the age of 1 month was fed milk mixtures in stead of mother s milk growth and development were normal On the day of admission a tender massing occurred in the right half of the scrotum The temperature rose up to 38 C An operation was performed at which the epididymis was found to have thickened throughout cauda epididymis was the site of a solid infiltration the size of a hazelnut from which a biopsy was taken The testis was normal but the funicle had thickened and was oedematous The biopsy showed non specific inflammatory changes with few eosinophil cells and normal vessels but the epithelium of the ductules was damaged

Seven days after the operation the patient suddenly had several episod s of vomiting and his faeces were of a watery consistency Within a few hours he became dehydrated and went into sho k but after parenteral application of fluid the cond tion improved A similar ep sode occurred 7 days later and like the first immediately upon intake of Eledon Since several provocat ve tests using mother s milk had released identical reactions the condition was presumed to be allergy to milk for which reason the patient was fed soybean milk He did not either tolerate fruit which consequently was withheld from the diet It was not until 7 months after discharge from hospital that he came to tolerate milk and fruit At that time he was 1 year old he was of normal length but under weight by 3 kg

Laboratory tests SR 9 Leucocyte count 7 000/mm and normal distribution Eosinophil cell count which at admission had been 63 rose up to 695/mm after the first acute episode Thrombocyte count 930 000/mm determined at a time when the patient was satisfactorily hydrated values normalized however within 2 weeks Microscopy of urine normal findings Culture of urine no growth In addition the following tests showed normal conditions Parotitis complement fixation test fructose saccharose and lactose tolerance tests sweat test fractionated cortisol analysis serum electrophore is GO and GP transaminase alkaline phosphatase galactose 1 phosphat uridyl transferase culture of blood and spinal fluid Urine was examined with a view to pregnantriol amino acid chromatography and owl-eye cells Faeces was examined for pathogenic intestinal bacteria and vira Intravenous urography was performed as well as radiography of the stomach and the intestinal passage

Ca e 4

A boy aged 2 months was hospitalized on the suspicion of left-sided orchitis Pregnancy and parturition had been normal Weight at birth 3 000 g He was breast fed for 5 weeks and subsequently fed milk mixtures His development was satisfactory and he was in good health until the day before hospitalization when he became unquiet and his temperature ro e up to 38 2 C As the left half part of the scrotum was swollen and tender an operation was performed When the scrotum was opened large volumes of a tarnished fluid were evacuated Epididymis was found to the site of considerable inflammatory processes involving marked reddening oedema and moderate fibrin coating whereas the testis and the funicle were of normal appearance The biop y obtained did not contain sufficient tissue for a diagnosis to be established by microscopy

Laboratory tests SR and leucocyte count normal findings Microscopy of urine normal findings Culture of urine no growth Intravenous urography normal findings Culture from the epididymis showed the presence of Escherichia coli

Case 5

A 5 week old boy was hospitalized to be observed for a left sided torsion of the testis Pregnancy and parturition had been normal Weight at birth 4 350 g He had been in good health until the day before hospitalization when he began to scream incessantly and his parents noticed a reddening and swelling of the scrotum The temperature was normal The physical examination revealed an increased content in the scrotum and a thickened funicle On the suspicion of

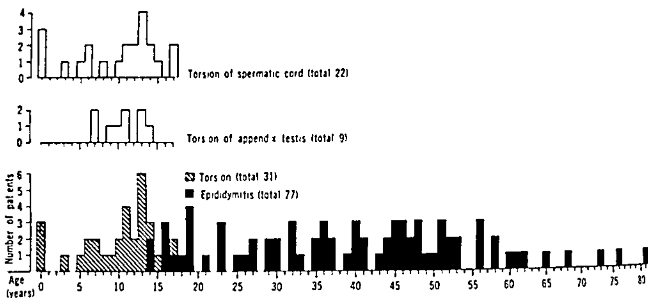


Fig 1 Comparison of age distribution in patients with torsion of the spermatic cord torsion of appendix testis and epididymitis studied by Amar & Chabra (1)

torsion of the testis an operation was performed at which the membranes were found to be oedematous cauda epididymis was the site of an infiltration the size of a hazelnut otherwise the epididymis and the testis were of normal appearance The biopsy showed changes similar to those observed in case 3 although they were more marked

Laboratory tests SR leucocyte count and differential count normal findings No eosinophilia Parotitis complement fixation test and Emni test negative No evidence of coxsackie virus nor of echo and poliomyelitis vira in faeces Microscopy of urine normal findings Culture of urine no growth Intravenous urography normal findings

Case 6

The patient was a 5 year old boy in whom epididymitis developed after a right sided orchiopexy because an infected nylon suture had penetrated the tip of cauda epididymis

DISCUSSION

Reports concerning the incidence of epididymitis in children in the prepubertal age group vary greatly During a 10 year period no more than 11 children with epididymitis were admitted to the Children s Hospital in Columbus Ohio (6) The diagnoses were verified at operation or by positive cultures of urine Among 610 patients examined by Mittemeyer et al (12) only eight were children Other authors who examined large series of patients did not find any cases of epididymitis among children In a Danish survey (8) of a series comprising 32 patients only one was a child

The clinical diagnosis may be difficult to establish and often it will not be arrived at until an operation is performed Usually the lesion will be regarded as torsion of the testis Both lesions may be responsible of pain in the scrotum reddening and oedema of the skin together with turgidity and tenderness of the scrotal content It applies as a general rule that torsion of the testis will be associated with severe pain but normal temperature which is in contrast with epididymitis In childhood however, symptoms may be deceptive and neither the intensity of pain the temperature or the SR and leucocyte count are suitable for differential diagnostic purposes For instance torsion of the testis to occur during the neonatal period need not at all be accompanied by pain (6) The age of patients may provide certain clues Fig 1 illustrates the age distribution characteristic of epididymitis and torsion of the testis While epididymitis mainly is seen in patients in the postpubertal age group torsion of the testis will generally be encountered in children aged 10 to 15 years although it may also occur during the neonatal period even during intra uterine life upon which the lesion will be manifest in the child at birth (1) As torsion of the

Table 1 *Material*

Case no	Age	Diagnosis	Microscopic findings
1	6 years	Epididymitis Allergic purpura (Schönlein Henoch)	Marked eosinophilia Marked vascular necrosis Normal epithelium in ductuli
2	5½ years	Epididymitis Allergic purpura (Schonlein Henoch)	Light eosinophilia Light vascular necrosis Normal epithelium in ductuli
3	3½ months	Idiopathic epididymitis Chronic digestive disorder (milk allergy?)	Unspecific inflammation Necrosis of epithelium in ductuli
4	2½ months	Pyogenic epididymitis (coli)	Biopsy specimen too small
5	5 weeks	Idiopathic epididymitis	Unspecifi inflammation Necrosis of epithelium in ductuli
6	5 years	Postoperative epididymitis	

testis often may result in atrophy of the testes *emergency* operation is generally indicated with a view to escaping this (1 3)

Torsion of appendix testis gives similar al though more moderate symptoms Tumours will *not initially* give either pain or oedema Hydro celes are pellucid they involve no pain and the scrotal content *is not increased* Allergic or idiopathic oedema of the scrotum will often produce *considerable* oedema the scrotal con tent will *remain normal* and the condition may often develop as a *complication* in infections of the upper respiratory tracts In cases of haematoceles and incarcerated inguinal hernia the anamnesis may serve as guidance

Orchitis may occasionally be accompanied by epididymitis (1) Such orchitis *may be ex* cited by parotitis virus coxsackie *virus* vari cella or mononucleosis but it is rarely *seen in* children One case of epididymitis secondary to parotitis but absence of *orchitis is on* record (5) Another although rare causative factor is gonorrhoea which was demonstrable in 2 of adult patients with epididymitis (12) The *aetio logy* of epididymitis remains obscure in more than 50 of all cases however (8 12) An epidemic of such idiopathic epididymitis is on record (14)

The aetiology of the disease is a much de bated question (4 8 11 12 14 15 16) Campbell (4) emphasizes that infection via the urinary tract may be a significant aetiological factor Mittemeyer et al (12) observed that 20 % of their patients with epididymitis had a concurrent infection of the urinary tract but only 2 of the patients were found to have malformations of the urinary tract Eegholm (8) found similar conditions he declared that haemorrhagic cystitis also might be a common finding Similar features may apply to children (1 6) A report appeared in 1969 according to which epididymitis had been diagnosed in three children in the prepubertal age group (2) vesico-ureteral reflux and other malformations of the urinary tract were demonstrable in all three children

It is still an object of much discussion how an infection may spread to epididymis but there is every probability that it may proceed via lymphogenic or more rarely haematogenic routes (15 16) Reflux of infected urine through the deferent duct occurs exclusively if the latter is dilated and without tonus for instance as a sequela of protracted infection (16) An in tensified intravesical pressure will under normal conditions result in an intensified tonus in the neck of the bladder and occlusion of the ejacula tory duct just as at the ureteropelvic conjunc tion (11) The ciliary function in the ejaculatory duct serves also to prevent reflux of urine Ligature of the deferent duct in patients with recurrent epididymitis does not lower the in

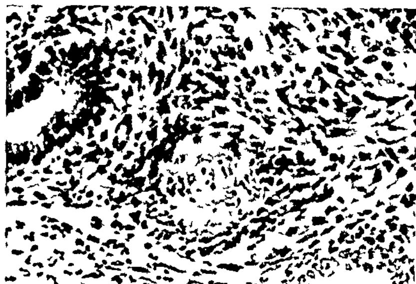


Fig 2 Marked vascular necrosis and normal epithelium in ductuli epididymis (Case 1)

cidence of recurrences and epididymitis is a common occurrence in vasectomized patients (16)

Infection of the urinary tract did not in any case accompany the epididymitis in the patients discussed here, such infection was not either manifest prior to admission or during the interval between hospitalization and follow up Intravenous urography showed normal conditions in five of the patients, one patient was found to have double kidney anlage on one side but no other anomalies This patient was the only one in whom cysto urethrography of micturition was done, but reflux was not observed In four of the children the SR or the leucocyte count remained normal while the SR was moderately elevated in two It is remarkable that three of the children, including the two with Schonlein Henoch s purpura presented increased thrombocyte counts over longer periods Patients suffering from the latter disease have usually normal thrombocyte values According to a recent publication thrombocytosis seems to be a common occurrence in patients with rheumatoid arthritis (10) The significance of this postulation remains to be defined but the phenomenon may be linked up with an immunological process

The patients were examined after intervals covering from 6 months up to 3 years after hospitalization the scrotum had normalized in all cases and there had not been any recurrences

The microscopic picture of epididymis in the two children with Schonlein Henoch s purpura was distinctly different from that of the other



Fig 3 Unspecific inflammation with necrosis of epithelium in ductuli epididymis (Case 5)

patients (Table 1) The vascular wall was the site of necrosis there was marked oedema of the wall and the endothelial cells were partly destroyed The vascular wall and the stroma were sites of inflammatory infiltrations composed of polymorphonuclear leucocytes with numerous eosinophil cells as well as of lymphocytes and plasma cells The columnar epithelium in the ductules was well preserved The changes were similar to those known from Schonlein Henoch s purpura they were most pronounced in case 1 (Fig 2) less pronounced in case 2 The other two biopsies revealed non specific inflammatory changes but well preserved vessels damaged epithelium in the ductules and inflammatory infiltration in the stroma with few eosinophil cells These changes were most pronounced in case 5 (Fig 3) less pronounced in case 3

Whenever a boy is found to have a painful and swollen scrotum torsion of the testis or incarcerated hernia should first be suspected and as a rule surgical intervention will be indicated Microscopy and culture of urine should be performed and if the diagnosis of epididymitis is verified the urinary tracts should be examined with a view to malformations If the patient is afflicted with a concurrent Schonlein Henoch s purpura there is every probability that it is a matter of an epididymitis involved as a complication in this disease

SUMMARY

The case histories of six children with epididymitis are submitted In 2 cases the epididymitis formed part of Schonlein Henoch s purpura and microscopy of biopsies from the epididymis showed changes characteristic of the latter disease In 1 case the epididymitis was of suppurative nature and in 1 case it represented a complication following orchiopexy In the remaining 2 cases the aetiology of the disease remained obscure

Infection of the urinary tract was not observed in any of the cases and the urinary tract was normal in all except one in whom double anal arc of one of the kidneys was demonstrable Three of the patients including the two with Schonlein Henoch s purpura had thrombocytosis The aetiology and the differential diagnoses are briefly discussed It is emphasized that the suspicion primarily should be focussed on torsion of the testis or incarcerated hernia whenever a boy is found to have a tender and swollen scrotum it is emphasized also that the two latter diagnoses as a rule can be excluded by operation

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CASE REPORT

A CASE OF PRADER-WILLI SYNDROME IN A GIRL WITH A SMALL EXTRA CHROMOSOME

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In 1956 Prader Labhart and Willi described 9 cases of a syndrome comprising mental deficiency short stature obesity hypogonadism and muscular hypotonia A tendency to diabetes (9) and acromicria (6) were subsequently added to the list of characteristic clinical features Since these reports a variety of other findings have been described in published cases which have been extensively reviewed and discussed (2 4 7 11) We are aware of 109 recorded examples of the syndrome of which 57 had been karyotyped Six of these are reported to have distinct chromosomal abnormalities of different kinds one with an extra Y chromosome (5) another with an additional G chromosome (12) a mosaic with D/E translocation (12) a D/D translocation (1) a mosaic D trisomy (10) and a mosaic G/G translocation (2) Further instances of chromosomal aberrations in persons with the Prader Willi syndrome may help to clarify the obscure relationship of such aberrations to the syndrome We therefore report an example of the syndrome in a patient with a small extra chromosome

CASE REPORT

J O (II) (a female born 1949) is the youngest of five surviving children (see Pedigree in Fig 1) The mother s first pregnancy (II) terminated in a stillbirth at 36 weeks and the third and fourth resulted in twin births with one twin in each pair stillborn and malformed (II and II) Unfortunately no information is available on the type of malformations in these two individuals J O s surviving sibs are all apparently normal though her sisters (II and II) did not menstruate until 15–16 years of age Her mother s menarche occurred at the age of 17 years followed by infrequent periods and marked gain in weight for the next 2 years The father of the proposita died at 65 years of pneumonia he had one son by a previous marriage The ages of the mother and father at the birth of the proposita were 44 and 63 years respectively There is no history of consanguinity in the family

J O was born at term by breech delivery after a rapid labour The pregnancy was uneventful apart from hypertension The infant weighed 2 800 g was hypotonic did not cry and was unable to suck Feeding difficulties persisted neonatally and the child lost weight Her condition appeared to improve thereafter However when she started school at 5 years she was found to be mentally retarded and was transferred to a training centre Increasing obesity was noted at 8 years and she developed a voracious appetite The development of secondary sexual characteristics was delayed

J O was referred to us at the age of 17 years (see Fig 2) for investigation of her amenorrhoea and obesity She was found to be mentally retarded with an estimated I Q of 40 and of a pleasant disposition She was short (height 140 cm) with marked generalized obesity (weight 72 3 kg) particularly affecting the cheeks chin lower trunk and legs The head was small and rounded with the following measurements circumference 520 mm length 172 mm breadth 142

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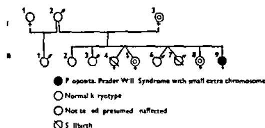


Fig 1 Pedigree of family

mm height, 120 mm cephalic index 0 83 She was myopic and had a small mouth high palate and grossly hypoplastic and carious teeth She was hypotonic the legs showed genu valgum and she walked with a lumbering gait Other features noted were acromicria hypogonadism abdominal striae retracted nipples and scanty axillary and pubic hair She had not yet menstruated

Chromosomal findings

Karyotypes prepared from cultures of p riph eral lymphocytes and skin fibroblasts showed a diploid number of 47 chromosomes in all cells examined The extra chromosome was consistently found to be slightly smaller and more metacentric than the chromosomes of the G group (see Fig 3) In some cells the additional chromosome showed satellites and satellite association Apart from occasionally dimorphic number 16 chromosomes no other peculiarities were detected Karyotypes from lymphocyte cultures taken from other available members of the family appeared to be normal The findings are summarised in Table 1

Other investigations

The urinary follicle stimulating hormone level was 6 mouse units/24 hours A glucose tolerance curve showed mild intolerance to glucose Radiographs of the hands and wrists indicated a normal bone age D rmatoglyphic examination r vealed no notable differences between the proposita and other members of her family

DISCUSSION

The main findings in the present case are compared, in Table 2 with those commonly reported in previously published cases of Prader Willi syndrome Clinically she appears to be a typical example of the syndrome despite the presence of the extra chromosome

Cases of the syndrome are more likely to be recorded if an associated chromosomal aberration is present Nevertheless the occurrence of 7 reported instances including the present one of chromosomal abnormality among 58 karyotyped patients with the syndrome is impres



Fig 2 Proposita at 17 years of age

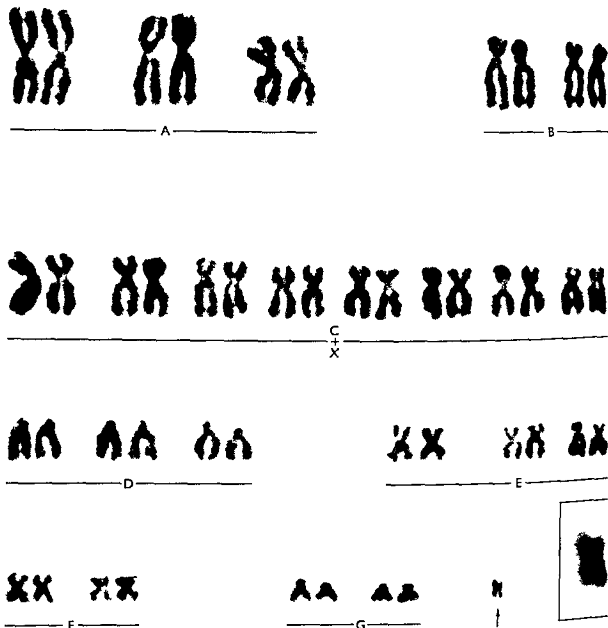


Fig 3 Karyotype of propo ita from a leucocyte cul ture showing 47 chromosomes The extra chromosome is arrowed with an enlargement adjacent

sive By contrast only 10 of 1 788 live born babies studied by Court Brown & Smith (3) showed definitely abnormal karyotypes The identity of the extra chromosome in our patient is uncertain If it is a fragment as seems likely the presence of satellites implies an origin from one of the D or G group of chromosomes The other examples of the syndrome reported to show chromosome abnormalities involve the D or G group in 5 cases (1 2, 10 12) and a Y chromosome in one (5) Although there is no consistency in these findings it may be im portant that acrocentric chromosomes are most commonly involved

Though a genetical basis for the Prader Willi syndrome has received consideration (4)

Table 1 *Chromosomal findings in proposita and relatives*

Case	Pedigree no (see Fig 1)	Culture	Cells counted	Karyotype	Comments	
Proposita	II	F	30	47/XX	Extra small satellited metacentric	
Proposita	II	L	30	47/XX	Extra small satellited metacentric	Occasional dimorphic no 16s
Mother	I	L	30	46/XX	Normal female karyotype	Occasional dimorphic no 16s
Brother	II_3	L	40	46/XY	Normal male karyotype	Occasional dimorphic no 16s
Sister	II	L	30	46/XX	Normal female karyotype	Occasional dimorphic no 16s
Sister	II	L	30	46/XX	Normal female karyotype	Occasional dimorphic no 16s

L = Leucocyte culture F = Fibroblast culture

Table 2 *Main clinical findings in proposita compared with those commonly recorded in published cases of Prader Willi syndrome*

Reported findings	Findings in proposita
Mental retardation	+
Short stature	+
Obesity (especially affecting lower trunk legs cheeks and chin)	+
Hypogonadism	+
Hypotonia	+
Acromicria	+
Abnormal glucose tolerance	+
Birth weight below 3 000 g	2 773 g
Neonatal feeding difficulties	+
Excessive appetite in childhood	+
Small head	+
Ocular anomalies	− (myopia)
Hypoplastic carious teeth	+
High arched palate	+
Micrognathia	−
Genu valgum	−
Delayed development of secondary sexual characteristics	−
Urinary gonadotrophins increased or decreased	Decreased
Retarded bone age	− (X ray hand and wrist)

Most characteristic features

the aetiology of the syndrome is as yet obscure Clarification of the possible significance of the findings mentioned above must await the accumulation of further data

SUMMARY

A characteristic case of Prader Willi syndrome is reported in a girl who showed additionally the presence of a small extra chromosome possibly a fragment of a D or G group chromosome Available members of the proposita s family were found to be phenotypically and cytologically normal The case is compared with those previously reported

ACKNOWLEDGEMENTS

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CASE REPORT

GIANT HAEMANGIOMA WITH A DISORDER OF COAGULATION

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University of Goteborg Sweden

Haemangioma is rarely complicated by dis orders of blood coagulation Such a complica tion was first described by Kasabach & Merritt (21) in 1940 since when about 80 cases have been reported (20 35–38) About 80% of these cases have been seen in children below 1 year

It was not until some 10 years ago that coagulation disorders attending haemangioma began to receive serious attention Of 19 cases (3–5 7–9 20 35 39 41 42 45) studied extensively thrombocytopenia (< 100 000) oc curred *in* 17 a decrease in factor V (< 80) in 10 and a decrease of factor VIII (< 60) *in* 5 A low fibrinogen concentration (< 260 mg/100 ml) was found in 17 cases and in creased fibrinolysis in 7 Platelet and fibrinogen turnover studies have been performed in a few cases Thus Blix & Aas (7) found a rapid dis appearance of ^{1}Cr labelled platelets from the blood and an uptake of the activity by the tumour Using ^{1} I labelled fibrinogen Wochner (43) demonstrated a high disappearance rate of fibrinogen which was normalised by ad ministration of heparin Hillman (19) reported an increased uptake of labelled fibrinogen over the haemangioma even when allowance was made for the increased local blood volume due to the tumour

The rapid disappearance of the labelled plate lets and the lab lled fibrinogen in these cases and the low platelets factor V and fibrinogen values reported in several other cases have often been interpreted as manifestations of intra vascular coagulation in the haemangioma with consumption coagulopathy as a result The in creased fibrinolytic activity recorded in some cases has also been regarded as secondary to the intravascular coagulation process Such mechanisms as mechanical destruction of the platelets in the tumour and destruction of the fibrinogen due to fibrinolytic activators pro duced by the tumour i e mechanisms other than intravascular coagulation have so far not been considered in the causation of the coagula tion disorders complicating haemangioma

This paper reports on a case of haemangioma with a disorder of the coagulation studied re peatedly during 2 years with various methods including the estimation of the turnover of platelets and fibrinogen with the aid of radio isotopes

This investigation was supported by grants from the Swedish Medical Research Council (B70-19X 87-06C)

CASE REPORT

The patient was a girl born in September 1967 The parents appeared healthy and pregnancy and delivery



Fig 1 Haemangiomatous arm at March 1970

had been uncomplicated. Nothing *remarkable* was noticed until the child was 3 *months* when a small blue patch was observed on the *left elbow*. *This* discoloration grew rapidly in size and within 3 weeks the entire arm was *involved by* a *blue red* tumour extending down to the *wrist*. *Small* petechiae had by then appeared on the neck and chest. *On first* admission to hospital (Lund January 1968) *the platelet* count (20 000 platelets/mm³) and the haemoglobin concentration (8 *g*/100 *ml*) were *low*. A biopsy specimen showed a picture resembling that of Kaposi's sarcoma but without convincing signs of malignant cells. The histological diagnosis was haemangiomatous. Angiography showed a soft tissue tumour with early shunting to venous cavities. Subsequent examination of the bone marrow showed an increased number of megakaryocytes. Roentgen irradiation of the haemangioma in January 1968 with a total dose of 800 R produced no demonstrable effect.

By the time of admission to Malmo General Hos-

Table 1 *Coagulation studies*

	May 1968	April 1969 Healthy arm	April 1969 Angiom arm	May[a] 1969	November[a] 1969	March[a] 1970	Normal range
Platelet number per mm³	22 000	28 000	20 000	74 000	20 000	30 000	150 000–400 000
Bleeding time							
Duke min	13	11			10	10	1–4
Ivy min	>30						6–15
Coagulation time							
glass min	9						8–14
plastic min	26						15–25
One stage prothrombin time sec	15	*16*	*12*	*14*	16		14–16
Recalcification time sec	157	125	135	152	140	180	120–180
P & P	90	*114*	114	120	105	79	80–120
Factor V	100	75	80	82	106	104	80–120
Factor VIII	113	197			*160*	82	60–140
Fibrinogen g/100 ml	0.31	0.20	0.14	0.24	0.24	0.30	0.26–0.34
Plasminogen							60–140
clot method	88	120	103	*60*	*104*		
immunochem method	88		90				
α_2 *macroglobulin*	227	220			233		
Fibrinolytic activity on unheated plates							
plasma mm²	30	35	0	18		0	0–50
resusp euglob prec mm²	40	125	88	66		10	0–70
Fibrinolytic split products mg/100 ml	3	3.9	6.4	1	1	Traces	0

[a] During treatment with EACA

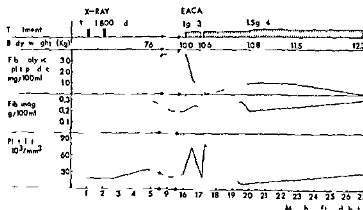


Fig 2 The course of the disease

pital in May 1968 the haemangioma had involved half of the upper arm and two thirds of the lower arm The arm was thickest in the region of the elbow The skin was somewhat uneven relatively warm purplish red and hairs were growing sparsely from its surface The arm felt abnormally firm to hard The arm was flexed 60 and stiff About 2 cm above the elbow joint the affected arm measured 24 cm in circumference compared with 14 5 cm on the other side Haemoglobin 10 6 g/100 ml Haptoglobin 186 mg 100 ml Other routine laboratory studies revealed nothing remarkable During the rest of the patient s stay in hospital the haemoglobin level was about 1 g/100 ml The coagulation of the blood was studied repeatedly (Table 1)

Owing to its increased vascularity the affected arm grew quicker than the contralateral arm Thus 9 months after onset the humerus was 9 mm the ulna 8 mm and the radius 7 mm longer on the affected s d

Since the patient showed high fibrinolytic activity in the tumour tissue and split products in the blood treatment was started with EACA (Epsikapron®) in a dose of 1 5 g 3 in April 1969 During treatment the haemangioma decreased peripherally and the d scoloration of the skin became less intense At the same time the platelet count increased (Table 1 Fig ?) After the patient had received EACA at home for 1 month the preparation was unintentionally with drawn Three days later bleed ng occurred in the haemangioma and the patient was re admitted to hospital On admission it was found that the platelet count had again fallen Resumption of treatment with EACA rapidly controlled the bleed ng the platelet count increased though only temporarily (Fig 2) During continued treatment with EACA the coagula tion of the blood w s examined on 3 occasions viz 19 ?? and ?7 months after the onset of the disease (Fig ?, Table 1) The size of the haemangioma suc cessively decreased more in area than in thickness

The circumference of the arm ? cm above the elbow joint was thus still 24 cm but the tumour now in vol ed only one third of the lower arm and barely one third of the upper arm The corresponding circum ference on the right side had now increased to 15 5 cm The skin was somewhat purpli h red but did not feel abnormally warm The arm was still as firm as before Some areas of normal skin were seen on the flexor side of the tumour (Fig 1)

The roentgenological difference in length of the humerus had now increased to 15 mm while that of the ulna and radius had decreased to 5 mm and 3 mm respectively

The physical development of the child was other wise normal

LABORATORY STUDIES

Methods

Platelet count were made by the method of Bjork man (6)

Bleeding time (Duke s technique) was determined bilaterally Standardized haemolets (sera Sharp blood lancets Propper MRG Co New York) were used The bleeding time was also determined by the method of Ivy as modified by Nilsson et al (31)

Prothrombin factor VII and factor X were meas ured by the P & P method of Owren & Aas (32) factor V principally according to Wolf (44) and factor VIII according to Nilsson et al (28)

Fibrinogen was determined in the way described by Nilsson & Olow (29) Only fibrinogen values for blood collected with EACA are given

Plasminogen was measured both by a clot method (30) and by an immunochemical method (16)

The α_2-macroglobulin concentration was determined by the method of Ganrot (5)

Fibrinolytic activity of plasma and resuspended euglobulin precipitate was measured on unheated bo vine fibrin plates as described by Nilsson & Olow (29)

Fibrinolytic split produ ts were determined with the immunochemical method of Nilehn (27) The analysis were performed on serum samples obtained from blood coll cted in tub s containing EACA

The plasminogen activator content of the venous wall was measured as the amount in a roughly 0 5 cm long biopsy specimen obtained from the haemangioma with the patient under general anaesthesia The fi

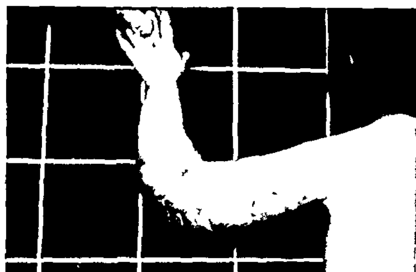


Fig 1 Haemangiomatous arm at March 1970

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Factor VIII	113	197			160	82	60–140
Fibrinogen g/100 ml	0 31	0 20	0 14	0 24	0 24	0 30	0 76–0 34
Plasminogen							
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Fibrinolytic activity on unheated plates							
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resusp euglob prec mm²	40	125	88	66		10	0 70
Fibrinolytic split products mg/100 ml	3	3 9	6 4	1	1	Traces	0

[a] During treatment with EACA

gioma The haemangiomatous arm was about twice as thick as the other arm and therefore contained a much larger amount of blood The values found for radioactivity on either side are therefore not strictly comparable There was however a continuous increase in activity over the haemangiomatous arm during the first 2–3 hours when the activity in the blood was decreasing This indicates that the higher activity over the haemangiomatous arm was due not only to the larger size of the arm but to a true uptake

The rapid disappearance of the platelets in the haemangioma may be explained either by a consumption of the platelets in the tumour due to formation of microthrombi and/or by

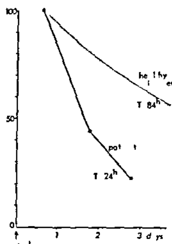


Fig 5 Disappearance rate of labelled fibrinogen in the patient and in healthy adults

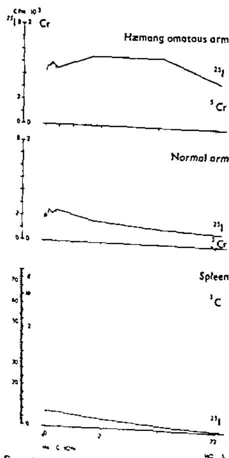


Fig 4 ⁵¹Cr activity over haemangiomatous arm normal arm and over the spleen after injection of labelled platelets

mechanical breakdown of the platelets in the dilated sinusoids in the haemangioma The finding of normal values for the various coagulation factors in association with a very low platelet count argues against a consumption syndrome

The parallel course of the uptake of Cr activity by the haemangioma and the spleen is however striking and suggests that the haemangioma behaves like the spleen It thus appears probable that some of the platelets are destroyed mechanically in the dilated sinusoids in the haemangioma On the other hand there was definitely some evidence of intravascular coagulation A moderate consumption of coagulation factors need not cause a coagulation defect for as known from turnover studies of fibrinogen (23) the body can compensate for an increased loss by an increased synthesis In

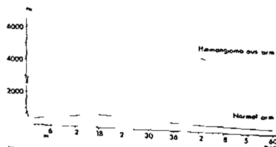


Fig 6 ¹²⁵I activity over haemangiomatous arm and normal arm aft r injection of labelled fibrinogen

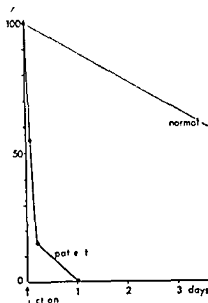


Fig 3 Disappearance rate of labelled platelets in the patient and in healthy adults

brinolytic activity was demonstrated with Pandolfi s modification of the histochemical method of Todd (40) and assessed according to the grading used by Pandolfi et al (33) In normal individuals the plasminogen activator content of superficial arm veins is 6–11 arbitrary units

Platelet turnover was estimated with the aid of ⁵¹Cr labelled (1) platelets from the patient s mother Fibrinogen was labelled with ¹²⁵I essentially according to the capillary jet technique of McFarlane (25) as modified by Blomback et al (11) but with the use of iodine monochloride (ICl) as the iodine source instead of molecular iodine (I) (26) The fibrinogen was prepared from plasma (10) from a few selected donors The radioactivity was determined (23) in a fibrin solution

External counting after injection of Cr labelled platelet and ¹²⁵I labelled fibrinogen respectively was performed with a collimator The activity was measured over the haemangiomatous arm over the normal arm and in the platelet study also over the spleen

RESULTS

The results of the coagulation studies are given in Table 1 and in Fig 2 On admission to hospital in May 1968 the patient had thrombocytopenia and prolonged bleeding time (Duke s and Ivy s technique) The fibrinogen prothrombin, factor V and factor VIII and plasminogen values were normal No increased fibrinolytic activity could be demonstrated in the circulating blood, which however contained fibrinolytic split products The plasminogen activator content of the walls of the tumour vessels in the biopsy specimen of the tumour was high (7 arbitrary units) On one occasion (April 1969) when samples were obtained from both arms the sample from the affected side contained less fibrinogen and more fibrinolytic split products than that from the contralateral side

The results of the platelet turnover studies are given in Fig 3 The activity fell to about 50% within 2 hours compared with 110 hours in normal adults Most of this activity was taken up by the affected arm (Fig 4) This uptake started early after the injection and parallel to the uptake in the spleen

Fig 5 gives the results of turnover studies with ¹²⁵I labelled fibrinogen which indicated a half time of about 24 hours compared with 84 hours in three healthy adults studied in this laboratory The uptake by the haemangiomatous arm was high and progressed during the first 18 hours after the injection (Fig 6)

Administration of EACA was followed by an increase of the platelet count and a decrease of the amount of split products in the blood (Fig 2) The fibrinogen level and the other coagulation factors remained largely unchanged After 1 month EACA was withdrawn and the platelet count fell but rose after resumption of treatment with the drug The platelet count afterwards gradually fell despite treatment with EACA (Fig 2) Only traces of split products were demonstrable in the last samples As pointed out above the haemangiomatous arm continuously decreased in size during EACA therapy (Fig 1)

DISCUSSION

The coagulation studies in this patient with a giant haemangioma of the left arm revealed severe reduction in the number of circulating platelets Judging from the turnover studies this thrombocytopenia was obviously due to a rapid disappearance of platelets The external counting indicated that the labelled platelets were to a large extent taken up by the haeman

gioma The haemangiomatous arm was about twice as thick as the other arm and therefore contained a much larger amount of blood The values found for radioactivity on either side are therefore not strictly comparable There was however a continuous increase in activity over the haemangiomatous arm during the first 2–3 hours when the activity in the blood was decreasing This indicates that the higher activity over the haemangiomatous arm was due not only to the larger size of the arm but to a true uptake

The rapid disappearance of the platelets in the haemangioma may be explained either by a consumption of the platelets in the tumour due to formation of microthrombi and/or by mechanical breakdown of the platelets in the dilated sinusoids in the haemangioma The finding of normal values for the various coagulation factors in association with a very low platelet count argues against a consumption syndrome

The parallel course of the uptake of Cr activity by the haemangioma and the spleen is however striking and suggests that the haemangioma behaves like the spleen It thus appears probable that some of the platelets are destroyed mechanically in the dilated sinusoids in the haemangioma On the other hand there was definitely some evidence of intravascular coagulation A moderate consumption of coagulation factors need not cause a coagulation defect, for as known from turnover studies of fibrinogen (23) the body can compensate for an increased loss by an increased synthesis In

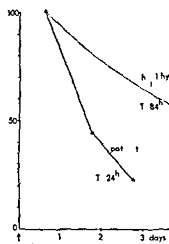


Fig 5 Disappearance rate of labelled fibrinogen in the patient and in healthy adults

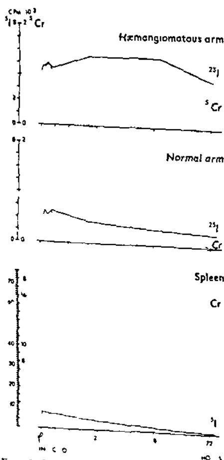


Fig 4 ^{51}Cr activity over haemangiomatous arm normal arm and over the spleen after injection of labelled platelets

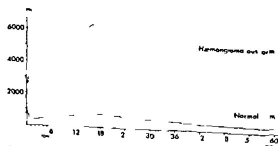


Fig 6 ^{125}I activity over haemangiomatous arm and normal arm after injection of labelled fibrinogen.

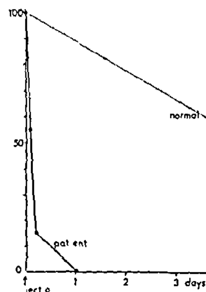


Fig 3 Disappearance rate of labelled platelets in the patient and in healthy adults

brinolytic activity was demonstrated with Pandolfi s modification of the histochemical method of Todd (40) and assessed according to the grading used by Pandolfi et al (33) In normal individuals the plasminogen activator content of superficial arm veins is 6-11 arbitrary units

Platelet turnover was estimated with the aid of ⁵¹Cr labelled (1) platelets from the patient s mother Fibrinogen was labelled with ¹²⁵I essentially according to the capillary jet technique of McFarlane (25) as modified by Blomback et al (11) but with the use of iodine monochloride (ICl) as the iodine source instead of molecular iodine (I) (26) The fibrinogen was *prepared from plasma (10) from a few selected donors* The radioactivity was determined (23) in a fibrin solution

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this patient the turnover rate of fibrinogen was relatively high but not as high as that of platelets Though no data are available concerning the turnover rate of these components in healthy children there is no reason to believe that they are significantly shorter than in adults The external counting indicated an uptake of fibrinogen by the haemangioma This uptake occurred later than that by platelets and seemed to continue for a longer time indicating the presence of a coagulation process in the haemangioma

The presence of fibrinolytic split products in the peripheral blood showed the occurrence of degradation of fibrinogen and/or fibrin The biopsy of the tumour showed high activity of activators of fibrinolysis in the walls of the small vessels in the angiomatous tissue Blood from the affected arm contained more split products and less fibrinogen than did blood from the other arm Taken together these findings indicated fibrinolysis in the haemangioma Activators must be released from the vessels and after activation of the fibrinolytic system precipitated fibrin and possibly also fibrinogen are degraded

The decrease in the amount of split products following treatment with EACA provides further evidence of involvement of a fibrinolytic component The use of heparin has been recommended in the treatment of the coagulation disturbances in patients with this type of haemangioma It has been claimed that heparin inhibits consumption of the coagulation factors (7, 12 19) But no effect on the structures of the haemangioma have been observed It might be mentioned that in a recent case of haemangioma with consumption of coagulation factors treatment including heparin therapy could not ward off a fatal issue (20)

We refrained from using heparin in our case because coagulation and turnover studies had indicated that the main cause of the thrombocytopenia in our patient was mechanical destruction of the platelets in the haemangioma and that intravascular coagulation played only a minor role and second because administration of heparin to patients with a low platelet count involves a high risk of bleeding. Third, the high fibrinolytic activity in the tumour vessels and the occurrence of fibrinolytic split products in the blood suggested rather the use of a fibrinolytic inhibitor in the treatment of this case We therefore decided to use EACA in order to inhibit the fibrinolytic activity in the affected arm and thereby possibly facilitate thrombotisation of the haemangioma Since this form of tumour often regresses spontaneously it is not possible to say anything definite about the effect of EACA except that it had no side effects On the contrary the tumour decreased in extent and the discoloration of the skin became less intense The girl had no serious bleeding episodes except during unintentional withdrawal of EACA

In cases of haemangioma with coagulation disorders we think it is worth while to try to find out to what extent the disorder is due to intravascular coagulation mechanical destruction of the platelets in the tumour and destruction of fibrinogen or fibrin by fibrinolytic activators produced by the tumour and to treat the patients accordingly

SUMMARY

The mechanism of the coagulation disorder in an infant with a large haemangioma of the right arm has been studied The disorder was characterised by thrombocytopenia prolonged bleeding time and the occurrence of fibrinolytic split products in the blood The levels of various coagulation factors were normal The mechanism of the disorder was further investigated by injection of ^{51}Cr labelled platelets which disappeared very rapidly from the circulation owing to the uptake by the haemangioma Injection of ^{125}I labelled fibrinogen revealed a considerable increase in disappearance rate which however was not as high as for ^{51}Cr labelled platelets Treatment with heparin in this case appeared inadvisable because the thrombocytopenia was found to be due mainly to mechanical destruction in the haemangioma

while intravascular coagulation played only a subordinate role. On the other hand the fibrinolytic activity in the tumour vessels was found to be high which together with the occurrence of fibrinolytic split products in the blood was considered to indicate treatment with inhibitors of fibrinolysis (EACA). During this treatment the angioma decreased *in size*. It would therefore appear advisable to investigate the mechanisms of the coagulation disorders in these patients before deciding upon treatment.

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Acta Pædiat Scand 60 235–238 1971

CASE REPORT

OCULODENTODIGITAL DYSPLASIA SYNDROME

KUTAY TAYSI BURHAN SAY TANJU FIRAT and GULFR GURSU

From the Departments of Paediatrics Opthalmology Plastic and Reconstructive Surgery Hacettepe University School of Medicine Ankara Turkey

Since the original description of this clinical entity by Lohmann in 1920 (7) additional cases from various parts of the world have been published (1 2 8 9 10) Oculodentodigital dysplasia syndrome (ODD) is characterized by a thin nose with hypoplastic alae microphthalmus microcornea and other ocular anomalies syndactyly and camptodactvly of the fourth and fifth fingers clinodactyly of the fifth finger and hypoplasia of the enamel (5) So far there have been 44 cases in 21 families described in literature including ours (11) The purpose of this report is to present a case where characteristic features of the ODD syndrome are present

CASE REPORT

The propositus a ?3 months old boy was a full term product of a normal pregnancy and delivery The mother was 24 and the father was 39 years old It was the first pregnancy of the mother she had two others subsequently one of which resulted in stillbirth and the other in an abortion The patient s growth and development were within normal limits

The parents were normal except that the father had a thin nose with moderate hypoplastic alae There was no parental consanguinity and the family history for eye and finger anomalies was negative

Clinical findings Physical examination revealed a well-developed boy with multiple congenital anomalies. His height was 90 cm (75 percentile) weight 13 kg (50 percentile) and head circumference 49 cm (50 percentile). The head was elongated vertically the hair was dry and brittle He displayed bilateral epicanthal folds hypotelorism small aperture of the eyelids bilateral microphthalmia microcornea and strabismus The nose was thin with marked hypoplasia of th alae and narrow nostrils (Fig 1) Bilateral syndactyly and camptodactyly of the fourth and fifth fingers and bilateral clinodactyly of the fifth fingers were noted (Fig 2) Dermatoglyphic studies of the finger tips palms and soles revealed no abnormalities Dental examination revealed two fractured teeth and very poor oral hygiene There was nothing to suggest that there was enamel hypoplasia

His motor and mental development were within normal limits for his age (Gessell developmental test) His responses during sound field hearing testing were around 20 decibel level This finding and the parental history were interpreted as an indirect evidence that his hearing was normal

Roentgenographic examination of the feet showed only two phalanges in the fifth toes bilaterally Metacarpal and metatarsal bones were broad and short In addition the middle phalanges of the fourth and fifth fingers were hypoplastic and there was medial angulation of the proximal interphalangeal joints of the fourth fingers bilaterally (Fig 3)

Chromosome analysis from cultured peripheral blood leukocytes was normal

DISCUSSION

The clinical and the roentgenographic findings in our case are typical of the ODD syndrome Tho e findings can be summarized in the following categories

Head Typical thin nose with hypoplastic alae and narrow nostrils epicanthal folds and hypotelorism constitute the characteristic facial appearance Microcornea is known to be a frequent sign (11) Microphthalmus is also fre

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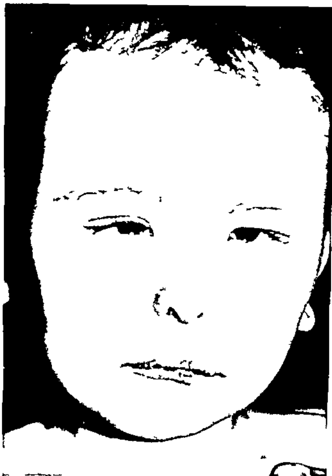


Fig 1 Facial appearance of the patient

quently seen and in some cases it it associated with small orbits (6) The lid aperture, interpupillary and inter canthal distances are reduced (5) Congenital cataract colobomas of the various ocular structures, strabismus and remnants of the pupillary membrane are frequently present (5, 8) In addition in our case a corneal opacity was noted which has not been described before

Hair is brittle, sparse and dry (5) Head circumference is usually normal Microcephaly was noted only in 2 cases in literature (6, 12) In most cases including ours motor and mental development were within normal limits

Dental findings Teeth show generalized enamel hypoplasia in most cases (2, 5, 8 10 12) However in a very few cases the teeth were noted to be normal In our case normal *primary dentition does not necessarily rule out* the future occurrence of enamel hypoplasia of the primary or the permanent teeth

Extremities Bilateral syndactyly and camptodactyly of the fourth and fifth fingers and clinodactyly of the fifth fingers are *found in* almost all cases

Genetics We found twentyone families with the ODD syndrome in the studied literature In six the inheritance pattern appeared to be autosomal dominant The rest were sporadic (11) X linked inheritance can be ruled out by the presence of an almost equal number of affected males and females and the presence of father to son transmission of the trait Besides the cases which showed autosomal dominant

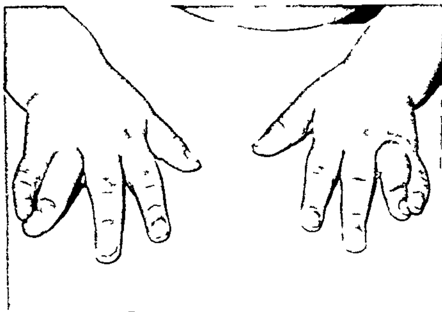




Fig 3 Roentgenogram of the hands howing hypoplasia of the middle phalanges of the 4th and 5th fingers bilaterally

inheritance there have been poradic ca es showing parental consanguinity The e cases might indeed be sporadic or they might be con idered to show autosomal recessive in heritance (3 4) However the generally ac cepted pattern of inheritance is an autosomal dominant (11)

The presence of sporadic ca e as well as familial ones with probably different inherit ance patterns can be explained in one of the following ways

1 This syndrome consists of several clini cally similar but basically different entities which show genetic heterogeneity

2 It shows autosomal dominant inheritance with incomplete penetrance and/or varying ex pressivity in some generations For example in our ca e the father s thin no e and hypo-plastic alae are suggestive of incomplete ex pre ssivity of the trait in the family The re mainder of the sporadic case is the result of new mutation which may eventually be proven to have dominant inheritance pattern

3 The sporadic cases are due to unknown mutagenic agents and are phenocopies

Analyses of the cultured peripheral blood leukocytes have been done in some of the cases in the literature none of which revealed any visible chromosomal abnormalities

SUMMARY

A 23 month old boy with typical findings of oculodentodigital dysplasia syndrome was pre sented and the inheritance pattern of this syn drome was discussed

ACKNOWLEDGEMENTS

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CASE REPORT

PROLONGED Q T INTERVAL AND CARDIAC SYNCOPES

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From the Department of Paediatrics G Rigshospitalet (University Hospital) Copenhagen and Department of Paediatrics Central County Hospital Nykøbing Falster Denmark

Cardiac syncopes in children due to malignant tachyarrhythmias are rare especially when no discernible organic heart disease is pre ent (19)

A syndrome consisting of syncopal attacks congenital deaf mutism and Q T prolongation in the electrocardiogram was de cribed by Jer vell & Lange Nielsen in 1957 (9) and then by several others (2 4 5 8 10–15)

Later syncopal attacks have been described in families with prolonged Q T interval but with normal hearing (1 6 7 12 16 18)

It is the aim of this paper to draw attention to this latter syndrome by reporting a case and the results of the family investigation

CASE REPORT

Boy born October 1967 He is the first of two chil dren the other sibling being a 17 months younger brother The pregnancy and delivery were normal and the birth weight was 3 200 g Growth and devel opment ha e been normal and he has had no child hood diseases

At the age of 9 months he started having syncopal attacks He usually started to scream hid his head in his hands became cyanotic on the l ps and then fell unconscious to the ground *He had stiff limbs* but no convulsions or incontinence When he recovered after ½ 1 minute he was lethargic and slept for about half an hour

H s attacks were sometimes provoked by excitement When he had a fever he used to have several attacks at short intervals. In October 1969 he had had a total number of about a dozen attacks.

Between the age of 15 months and 2 years he was admitted twice to the paediatric department of his regional hospital and once to the paediatric depart ment of the university hospital

Physical examination and chest roentgenogram were within normal limits Repeated electroencephalograms were entirely normal All laboratory tests including blood sugar serum electrolytes serum calcium serum magnesium serum uric acid and complete haemato logy were normal The acid base state was normal He had normal hearing

A low pulse rate (of about 70 per minute) was noted at times and the electrocardiogram was ab normal the T waves changed in configuration being negative at times and there was a constantly pro longed Q-T interval (Figs 1–2)

A 24 hours continuous ECG recording was obtained with telemetry and revealed no abnormal rhythm No ECG was obtained during an attack ECG during carotid sinus stimulation or while he was crying (per forming a Valsalva maneuver) wa unchanged

A diagnosis of cardiac syncopes in association with the prolonged Q T syndrome was made and he was digitalised during his last admission at the age of 2 years The Q T interval was hereby normalised (Fig 3) In the following 6 months he was doing well having only two short la ting attacks when the medi cation was not given by mistake The parents have been instructed in the techniques of resuscitation

Family investigation

Both parents are healthy The father was adopted and nothing is known about his family In the mother s family several members had migraine and a sister of the mother the maternal grandmother s brother and one of his sons had spells of convulsions in child hood supposed to be febrile convulsions (Fig 4) The maternal grandmother s mother was said to have had fainting spells when she was young she died forty years ago of unrelated disease

There was no history of congenital deafness or sudden death in the family

An electrocardiogram was obtained in 26 members

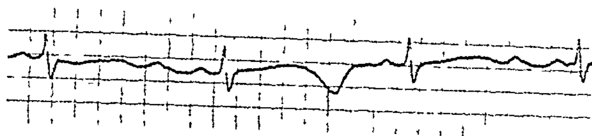


Fig 1 Electrocardiogram of our case (standard lead II) obtained in September 1969 The Q T *interval* is prolonged (R R 0 78 sec Q T 0 58 sec Upper normal limit 0 40 sec according to Alimurung et al *Circulation* 1 1329 1950) Paper speed 50 mm/sec, the distance between the distincter vertical lines being 0 10 sec

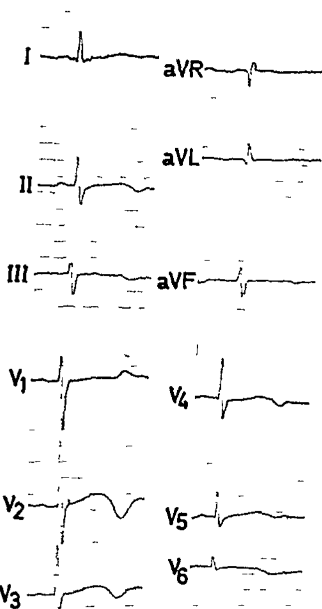


Fig 2 Electrocardiogram of our case obtained in September 1969 Note the bizarre T waves in the precordial leads

of the family (Fig 4) including all the mothers siblings and their children and including those mentioned above with previous convulsions A prolonged Q T interval was found in the patient s younger brother (Fig 5) and a borderline value in a cousin Neither of them have had syncopal attacks The maternal grandmother s father refused to have an ECG taken but an ECG from a hospital admission in 1964 showed a slightly prolonged Q T interval (R R 0 75 sec Q T 0 41 sec) the T waves were normal and he had never fainted

DISCUSSION

The combination of prolonged Q T interval and syncopal attacks is described with increasing frequency both in patients with congenital deafness and in patients with normal hearing The number of reported cases is approaching fifty but there are probably several unrecognized patients who are treated for epileptic spells Most of the cases have been described in members of the same family (1 5 6 7 9 13, 16 18) with autosomal dominent inheritance suggested for those with normal hearing (7) while autosomal recessive inheritance is claimed for those with congenital deafness (5)

In those case in whom an electrocardiogram was obtained during an attack the cardiac arrhythmia responsible for the syncope was shown to be ventricular fibrillation (2 7 8 10 16 18)

The etiology of the prolonged Q T interval is not clearly understood but is probably due to a metabolic abnormality in the myocardium which is not capable of normal repolarization (7) The mechanism by which the ventricular fibrillation may be precipitated has recently (7) been demonstrated to be 1) an increase in

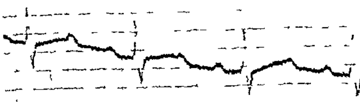


Fig 3 Electrocardiogram of our case (standard lead II) obtained in October 1969 after digitalisation The Q T interval is now normal

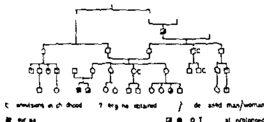


Fig 4 Pedigree of the family of our case Electrocardiograms were obtained in all living persons not marked with ?

the systemic blood pressure (pressure induced extrasystoles) 2) sinus tachycardia (when the impulse reaches the ventricles still in a depolarized state) or 3) an extrasystole produced in the supernormal phase of repolarization (R in T syndrome (3 17))

The syncopal attacks start in infancy or childhood the onset having been described between the age of 2 months and 12 years The attacks are usually provoked by exertional or emotional strain

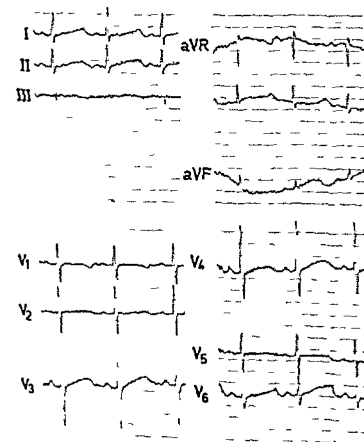


Fig 5 Electrocardiogram of our patient's younger brother obtained in October 1969 The Q T interval is prolonged (R R 0 50 sec Q-T 0 36 sec Upper normal limit 0 32 sec)

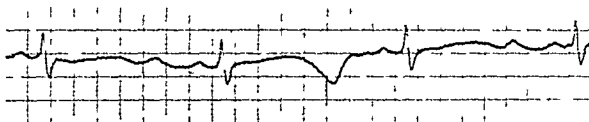


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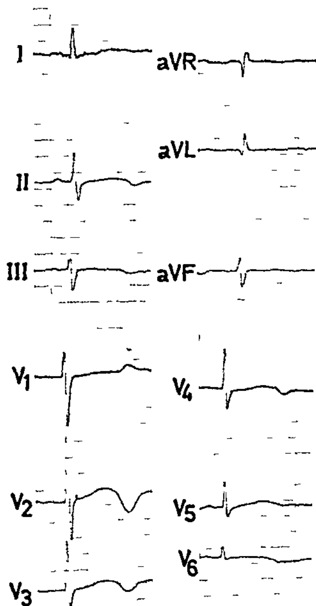


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PROCEEDINGS OF PAEDIATRIC SOCIETIES

DANISH PAEDIATRIC SOCIETY

Meeting Jan 14 1970

N J Brandt B Friis Hansen & J C Melchior *Diastrophic dwarfism*

Two patients with diastrophic dwarfism were demonstrated

The first was born by natural delivery following a normal pregnancy The birth weight was 3 000 g. At birth cleft palate and deformities of the extremities were demonstrated On both hands the fifth finger was crooked with radial angulation of the terminal phalanx and the first finger was very short Slight medial angulation of both femora marked curvature of both leg and severe pes equinus on both sides were also present The joints showed alternating contractures and hyperflexibility

The second patient was born at term by breech delivery following a normal pregnancy The birth weight was 2 800 g At birth the appearance was dysmature and the following were present large cephalhaematoma in the occipital region cleft palate short upper limbs hyperextension of th knees with dislocation of the patellae 60 posterior angulation of both tibiae and slight equino-varus deformity of the feet

Hitherto over 20 cases of this syndrome have been described in the literature The syndrome includes the following (1) deformities of the extremities (2) scoliosis (3) cleft palate and (4) deformed external ears The d formities of the extremities are particularly characteristic on account of the degree of twisting esp cially of the lower limbs All limbs are short and podgy and the epiphyses are particularly deformed The first metacarpal is practically invariably short and frequently deformed The disease is considered to be of autosomal recessive heredity

In the cases described here the family histories were negative All four parents had however been employed in departments of anaesthesia about the time of conception and the mothers had continued to work there during pregnancy

Ole Ortved Andersen & Knud E Pe ersen *An enuresis material from the Children s Hospital Fuglebakken*

An account is given of a material of 135 children (76 boys and 59 girls) aged 4–15 years who were admitted on account of enuresis to the Children s Hospital Fuglebakken Copenhagen

In an attempt to obtain a more differentiated picture and thus a possibility for more rational therapy of this very heterogeneous group of patients the material was subdivided into five groups

Group I Children in whom the bladder function had probably matured late These were selected according to the criteria of primary enuresis nocturna and as far as possible deep sleep Further children who slept very deeply with secondary and possibly both nocturnal and diurnal enuresis were included in this group None of these children exhibited the marked symp oms which were characteristic for Groups II and III Forty-one per cent of

The prognosis is grave as more than one third of the reported cases have died during an attack The danger of a fatal outcome seems to be diminished once the patients reach adulthood (10) Autopsy in a few cases conducted with special reference to the conducting system have shown consistent abnormalities (5)

Several antiarrhythmic drugs have been advocated as prophylactic treatment Best effect has been reported with digitalis which shortens the Q T interval (4 10), and with beta blocking agents (7, 18) Other measures are restraint from overexertion and emotional stress and instruction of the relatives neighbours, school teachers etc in resuscitation technique

As to our patient, there can be no doubt that he has cardiac syncopes in association with prolonged Q T interval though we have not recorded an ECG during an attack He is doing well on treatment with digitalis but should his attacks persist, we will change the medication to a beta blocking agent We have not been able to find any other member of the family with cardiac syncopes though prolonged Q T interval has been found in his brother and in a 5 year old ECG of his maternal grandmother s father

An ECG should be obtained in any child with spells of unconsciousness the diagnosis of prolonged Q T interval is easily made and prophylactic antiarrhythmic treatment is important in view of the grave prognosis Should the Q T interval be normal the child may still have cardiac syncopes and an exercise ECG should be obtained if possible (19)

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the children mainly boys, were classified in this group

Group II Children with *evidence of small* bladders These were selected on account of the symptoms of frequent micturition and urgency of miturition These should *electively have primary nocturnal enuresis* Children with secondary or possibly combined nocturnal and diurnal enuresis were *included in this* group provided they did not *present the symptoms of* Group III Ten per cent of the children mainly *girls* were *classified in this group*

Group III Children with emotional problems *Disturbances* of behaviour or environmental factors which were considered to constitute emotional *burdens were the* decisive criteria for selection The children in this group *should* have secondary enuresis but children *with* primary enuresis and pronounced behaviour disturbances were included *Forty per cent of the* children mainly girls qualified for this group

Group IV Children with malformations of *the urinary tract or* recurrent urinary tract infections Six per cent of the children mainly girls were encountered in this group

Group V Very immature children who were *mentally retarded* or psychoinfantile Three per cent of the children could be placed in this group

Knud E Petersen & Ole *Ortved Andersen* *Treatment of nocturnal enuresis with imipramine and related preparations A double-blind trial with a placebo*

The mechanism of the effect of imipramine on enuresis has not yet been elucidated In addition to its anti depressive effect the preparation has also an anti cholinergic effect In order to investigate the mode of action the authors investigated imipramine together with imipramine *N* oxide which has the same anti-depressive effect but is without effect on the autonomic nervous system and emepronium, an anti cholinergic preparation

In an out patient therapeutic trial the preparations were administered together with a placebo in randomized order employing a *double blind technique to 69 children* who had *previously been admitted to the* Children s Hospital *Fuglebakken* on account of enuresis and *who* were *still* wet at night The distribution of *the children in the* enuresis groups was as described in the previous lecture The dosage employed was 50 mg (corresponding to 1-2 5 mg/kg) administered 1 hour before bedtime This dosage appears to be equally *effective in* all age groups from 4 to 15 years Apart from tremor during imipramine therapy in 1 patient no side-effects necessitating withdrawal of treatment were registered

Thirty per cent of the children became dry following treatment with imipramine for 2 weeks Imipramine reduced the number of wet nights in the entire material to approximately *50%* of the incidence in a corresponding placebo period By contrast imipramine *N* oxid lowered the incidence only to 75% while emepronium had the same effect as a *placebo* In assessment of the results the protracted effect of the preparations was taken into consideration In certain children particularly those with *presumed* psychogenic causes (Group III) the incidence of enuresis could be reduced to 30% The incidence increased slightly, but the effect was still very pronounced after treatment for 3 months with imipramine spontaneous improvement being taken into consideration Following cessation of treatment the incidence of enuresis increased to the previous level

The anti-cholinergic effect is without significance The psychic effect of imipramine is of significance in connection with enuresis possibly in combination with an effect on sleep

O Steinicke *Imipramine treatment of enuresis A material from Christmas Seal Homes*

In order to assess the effect of imipramine (Tofranil®) on enuresis all children admitted during a period of 1 year on account of this condition to Christmas Seal Homes were in

cluded in a therapeutic trial with a placebo group In addition the children were followed up 3-4 months after discharge

A total of 225 children aged 4-14 years were treated These children had enuresis more than thrice weekly before admission Tofranil was administered to 110 and a placebo to 115 children Treatment was administered for 60 days Children aged 4-6 years received 10 mg children 7-9 years 20 mg and children 10-14 years received 30 mg The preparation was ad ministered 1-2 hours before bedtime in cases of nocturnal enuresis and in diurnal enuresis in corresponding doses in the morning Chil dren with both nocturnal and diurnal enuresis received medication both morning and eve ning In evaluating the results the latter group was assessed separately for both diurnal and nocturnal enuresis 23 patients in the Tofranil group and 31 in the placebo group being in volved These cases were thus added to both the purely diurnal and the purely nocturnal groups

Nocturnal enuresis comprised by far the larg est group and in 104 cases treated with Tofranil good improvement was demonstrated i e the incidence of enuresis was reduced to less than one third of the original incidence in 36% In comparison 107 cases were treated with the placebo and good improvement oc curred in 20%

On follow up investigation 3-4 months af ter the stay in the Christmas Seal Home good improvement had occurred in 29% of 94 pa tients treated with Tofranil as compared with 15% of 96 patients in the placebo group Cor responding figures in patients with diurnal en uresis were good improvement in 52% of 29 patients treated with Tofranil and 28% of 39 patients treated with the placebo and on follow up examination 26% and 14% respec tively

Thus both during institutionalization and on follow up examination the results from Tofra nil appear to have been somewhat better than from the placebo The author considers there fore that Tofranil should be considered as a therapeutic possibility in enuresis although in slightly larger dosages than those employed here and only for 2-3 months

Meeting Febr 11 1970

J Haahr & L Bohn *Uricult® A simple method of semi-quantitative culture from urine*

Uricult is a semi-quantitative object glass method of demonstration of bacteriuria elabo rated on the basis of the works of Naylor & Guttmann in 1967 An object glass is covered on one side with meat extract peptone agar and on the other with MacConkey s agar The glass is dipped into the specimen of urine and incubated at 35 C On the following day the number of bacteria are accessed by comparison with the accompanying scale Mid stream speci m ns of urine are employed or specimens ob tained by means of corresponding technique Uricult does not require refrigeration during transport

In Glostrup Hospital (Department of Paedi atrics and Central Laboratory) we have under taken a comparison between Uricult and ordi nary quantitative culture according to the loop method A total of 438 specimens were in vestigated and 121 of these were from children under the age of 15 years The period of in cubation was 18-20 hours Significant growth was obtained in 213 sp cimens i e at least 100 000 bacteria per ml by both methods In 12 cases lack of agreement was present as only one of the methods showed significant growth When compared with the clinical findings and microscopic examination of the urine it ap peared however that only two infections were not revealed by Uricult and similarly two in fections employing the conventional method

In 67 specimens with significant growth em ploying the loop method and Uricult at 35 the Uricult method employed at room temperature

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The mechanism of the effect of imipramine on enuresis has not yet been elucidated In addition to its anti depressive effect the preparation has also an anti cholinergic effect In order to investigate the mode of action the authors investigated imipramine together with imipramine *N*-oxide which has the same anti depressive effect but is without effect on the *autonomic* nervous system and emepronium an anti cholinergic preparation

In an out patient therapeutic trial the preparations were administered together with a placebo in randomized order employing a double blind technique to 69 children who had previously been admitted to the Childrens Hospital, Fuglebakken on account of enuresis and who were still wet at night The distribution of the children in the enuresis groups was as described in the previous lecture The dosage employed was 50 mg (corresponding to 1-25 mg/kg) administered 1 hour before bedtime *This dosage appears to be equally effective in* all age groups from 4 to 15 years Apart from tremor during imipramine therapy in 1 patient *no side effects necessitating withdrawal of treatment were registered*

Thirty per cent of the children became dry following treatment with imipramine for 2 weeks Imipramine reduced the number of wet nights in the entire material to approximately 50% *of the incidence in a corresponding placebo period By contrast, imipramine N* oxide lowered the incidence only to 75% while emepronium had the same effect as a placebo In assessment of the results the protracted effect of the preparations was taken into consideration In certain children particularly those with presumed psychogenic causes (Group III) the incidence of enuresis could be reduced to 30% The incidence increased slightly but the effect was still very pronounced after treatment for 3 months with imipramine spontaneous improvement being taken into consideration Following cessation of treatment, the incidence of enuresis increased to the previous level

The anti cholinergic effect is without significance The psychic effect of imipramine is of significance in connection with enuresis possibly in combination with an effect on sleep

O Steinicke *Imipramine treatment of enuresis A material from Christmas Seal Homes*

In order to assess the effect of imipramine (Tofranil®) on enuresis all children admitted during a period of 1 year on account of this condition to Christmas Seal Homes were in-

slight webbing between the fingers of the left hand The shoulder and elbow joints appeared normal and there was no evidence of pareses The hip joints were freely mobile and there were slight contractures in the knee joints

The feet were deformed on both sides but more so on the left side where the musculature of the leg was atrophic and a pronounced club-foot deformity was present On the right side there was complete dislocation of both the talo-crural and subtalar joints

The oesophagus would not permit passage of a probe and radiography confirmed the sus picion of atresia of the oesophagus No clinical evidence of cardiac or pulmonary disease was present The ordinary laboratory investigations for blood sugar haemoglobin serum calcium and microscopic examination of the urine re vealed normal findings On account of the low values for o stradiol excretion found in the ma ternal urine the infant s urine was investigated for fractionated 17 ketosteroids and values which were probably normal for the neonatal period were found

The infant died at the age of 93 hours Autopsy revealed

Increased quantities of cerebro-spinal fluid and agenesis of the corpus callosum Atresia of the oesophagus with fistula formation be tween the distal oesophageal segment and the trachea The left lung showed complete absence of lobulation No cardiac defects were present The gall bladder and the cystic duct were absent but a normal papilla of Vater was pres ent. A probe could be passed for ome cen timetres into the common bile duct which ap peared to disappear into the liver as a fibrous band No deformities were encountered in the urinary tracts and the vagina uterus salpinges and ovaries were normal for the age

The suprarenal glands were rather small but otherwise normal Microscopic examination of the kidneys liver and lungs showed normal conditions.

Chromosome investigation undertaken on cultured lymphocytes showed triploidy in all the 43 cells examined The sex chromosomes were XXX Autoradiography revealed that there were two late-synthesizing X chromosomes in 12 out of 22 cells examined and in nine cells one late synthesizing X was found

Blood type investigation suggested that it was most probable that the patient had re ceived two haploid chromosomes from the mother The results suggested that the patient had received both of the mother s rhesus alleles The child showed double-dose effect to anti M while the father had type N and the mother type M

Triploidy has previously only been described in two live born infants while it occurs rela tively frequently in spontaneous abortions where it is the third most common chromosome abnormality

Karen Thomsen *Diabetic coma without keton uria*

Hyperosmolar diabetic coma without ketosis is rare in childhood In June 1969 a male in fant aged 9 months was admitted to the Paedi atric Department, Glostrup Hospital with this condition He had high fever was dehydrated confused but not unconscious and pneumonia was present. The blood sugar was 1 232 mg/ 100 ml and the serum osmolarity was calcu lated as 359 mOsm/kg H^2O The standard bi carbonate was 12 mEq/l The urine remained free of acetone until 6 days after admission when the infant again developed high blood sugar and began to form acetone

The child was treated with insulin in rela tively small repeated doses and intravenous fluid in the form of isotonic saline bicarbonate and plasma On this treatment he recovered slowly and could be discharged later in good condition with 0 15 ml Insulin Rapitard daily

The syndrome of hyperosmolar diabetic coma without ketonuria is characterized by severe hyperglycemia hyperosmolarity and hy pertonic dehydration and the absence of keto sis Some cases but not all have lactic acido sis In this case the serum lactic acid was not determined In those cases that have been pub-

(20–23°C) rendered six false negative results but no false positive results If 10 000 *bacteria per ml had been employed as the limit of significant bacteriuria only one false negative* result would have been *found The material* does not permit conclusions to be *drawn regarding the employability of Uricult* at room temperature

Uricult provided equally reliable results as ordinary culture The method cannot replace microscopic examination of urine but provides a suitable supplement to this

S Sparrevohn & N J B Christiansen *A case of congenital myxoedema diagnosed on the second day of life by microanalysis of serum thyroxin*

The patient was a male infant the second of two siblings He was transferred to the Paediatric Department in Glostrup Hospital immediately after birth on account of a swelling as large as a fist on the anterior aspect of the neck and respiratory distress The birth weight was 3 400 g delivery occurred at term and the condition at birth was good in other respects The swelling was demonstrated to be a large goitre of cystic character The serum thyroxin on the second day of life was 8 9 μg/100 ml and 2 3 μg/100 ml on the fifth day of life

During the first 5 months of pregnancy the mother had consumed some special energy tablets and in this manner she had received approximately 7 g lithium and approximately 13 g potassium iodide The infant was given Eltroxin® therapy in a dosage of 0 1 mg daily and the goitre had disappeared entirely by the third week of life Radiographic examination revealed that Beclard's centres of ossification were absent at birth Protein bound iodine on the second day was 5 7 μg/100 ml

When the boy was 6 months of age Eltroxin treatment was withdrawn and follow up and control of serum thyroxin have since shown normal conditions No known cases of thyroid disease were present in the family The pronounced congenital goitre and myxoedema were probably due to abnormal sensitivity to iodine

This case history, similarly emphasizes the great value of microanalysis for determination of serum thyroxin Only small quantities of blood are required (100 μl serum) and the analysis has the great advantage that contamination with iodine is of no consequence (K Siersbæk Nielsen & J Mølholm Hansen, *Acta Pædiat Scand 56 141 1967)*

S Sparrevohn M Mikkelsen, E Niebuhr & K Henningsen *A live born patient with triploidy*

A newly born female infant was transferred from the Maternity Department to the Department for Neonates in Glostrup Hospital The child was the second of two siblings Her brother, aged 1 year, was healthy The mother had had an abortion in the third month in 1967 Apart from this there were no known abortions or children with malformations in the family The pregnancy had been completely uncomplicated with no signs of infection and the mother had not taken any form of medication nor contraceptive pills At 3 weeks and 11 days prior to the delivery oestriol excretion was low but the foetal movements and heart sounds were normal The foetus appeared to be abnormally small and 3 weeks before term caesarean section was performed The amniotic fluid was greenish the infant limp with a low Apgar scoring birth weight 1 600 g length 40 cm and circumference of head 33 5 cm The infant appeared very dysmature and had numerous malformations The head was strikingly large although not hydrocephalic the ears small and deformed protruding and slightly low set there was slight micrognathia bilateral blepharophimosis but the bulbi were normal The upper lip was rather long and thick The left hand was slightly deformed as if resulting from intrauterine pressure The hands were peculiar with long fingers of arachnodactyly type and the origins of the three radial fingers were more distal from the hand than the two ulnar fingers There was

face The lips became cyanotic and shortly afterwards h fell lost consciousness and had rattling respiration but no spasms After a few minutes he recovered consciousness and then slept for about half an hour after which he appeared completely normal again

The child was at first suspected of having epilepsy but all relevant investigations for epilepsy had negative results

On the other hand after the attacks the electrocardiogram was abnormal with very varying T waves which gradually become normalized while prolongation of the Q T interval persisted All electrolyte determinations showed normal findings Treatm nt with Digoxin was commenced The Q T interval returned to normal and the boy has been free from attacks for over 6 months

On investigation of the patient s family a prolonged Q T interval was demonstrated in his brother who is 17 months younger This boy has hitherto not had any cardiac syncopes

H T Lund Ib Transbøl & Ib Hornum *Hypercalcaemic sarcoidosis Report of a case in a girl a ed 13 years*

An unusual case of hypercalcaemia in a girl aged 13 years is described Her symptoms which consisted of fatigue loss of weight polyuria and thirst could all be attributed to the findings of lowered renal function and hypercalcaemia Investigation of the calcium metabolism revealed excessive hypercalcuria and metastatic calcification of the cornea (split lamp examination) and kidneys (renal biopsy) Tubular reabsorption of calcium (TRCa) and the cortisone test both suggested that the cause of the hypercalcaemia was non parathyroid

The Mantoux reaction was negative despite previous BCG vaccination The raised sedimentation rate and serum gammaglobulin and a muscle biopsy showing chronic inflammatory changes and multinuclear giant cells were the positive findings which suggested sarcoidosis as the only probable diagnosis

The patient was treated with prednisone after which the calcium metabolism and the renal function returned to normal

M Fjord Christensen & A J Therkelsen *A case of a XYYYY chromosome anomaly in a boy aged 2 ¹/₂ years*

The sex chromosome anomaly 49 XXXXY has been described in 42 patients since 1960 A clinical picture with oligophrenia certain mongoloid features hypogenitalism and slight skeletal malformations has been found to be typical for the anomaly

A new case of the 49 XXXXY anomaly in a boy aged 2 ¹/ years with the typical clinical picture is presented

Xg blood type determinations in the patient and his parents revealed that all four X chromosomes were maternal which suggests that the anomaly has developed by non disjunction in each of the egg cell s two meiotic divisions

The finding of a diabetic glucose tolerance curve is discussed in relation to the increased incidence of diabetes mellitus in patients with Klinefelter s syndrome and in patients with Turner s syndrome The genetic imbalance with either too many or too few X chromosomes may predispose to diabetes mellitus

Stig Sparrevohn *Four patients with saccharose malabsorption*

Meeting May 13 1970

B nt Friis Hansen *Directives for oxygen therapy in newly born infants* (Published in *Medicinsk Arbog* XIII Munksgaard 1970 pp 167–18?)

H H Seedorff *Retrolental fibroplasia*

B Djernes *Regional enteritis (Crohn s disease) in childhood*

Regional enteritis is a rare condition in childhood which is perhaps reported too seldom The symptoms are frequently generalized and

lished, the mortality was 50% Early diagnosis and intensive fluid and insulin therapy are important in order to lower the mortality

J Hahr & S Sparrevohn *Epididymitis in children*

Acute epididymitis is considered to be very rare prior to puberty The clinical diagnosis may be extremely difficult and as a rule emergency operation is indicated in order to exclude torsion of the testis In the majority of cases the etiology is unknown but in some cases urinary tract infection appears to be the cause

In the Paediatric Department in Glostrup Hospital 6 cases of epididymitis have been admitted in the course of the past 4 years Two of these developed in connection with Schonlein Henoch s purpura This complication has been mentioned only once previously in the literature One patient had suppurative epididymitis Another case occurred as a complication of orchidopexy while no explanation could be discovered for the 2 remaining cases In all of the cases the diagnosis was verified by operation None of the patients had had urinary infection prior to, during or after admission One child had a double renal system without other malformation and the remainder were all demonstrated by urography to have normal urinary tracts Three patients including the two with Schonlein Henoch s purpura, had thrombocytosis of long duration

Biopsy from the epididymis from the 2 patients with Schonlein Henoch s purpura showed pronounced necrosis of the vascular walls while the epithelium in the ductuli was normal The picture differed distinctly from the biopsy findings in the 2 children with idiopathic epididymitis where the changes were localized to the ductuli with destruction of the epithelium and inflammatory infiltration while the vessels were normal

Follow up examination from 6 months to 3 years after admission revealed normal findings on palpation of the scrotum in all the cases

Meeting March 11 1970

Discussion concerning the expansion of paediatrics in the future hospital services in Denmark

Meeting April 8 1970

Jørgen Kringelbach & Alf Wennevold *Prolonged Q T interval and cardiac syncope (Ward s syndrome)*

In 1957 Jervel & Lange described a syndrome in infants with congenital deafness consisting of cardiac syncopes and prolonged Q T interval accompanied by sudden death In subsequent years, this surdo-cardiac syndrome was described by various other authors from many different countries In 1963–64 reports of similar cases in children with normal hearing were published inter alii from Italy and Ireland (Ward)

A total of over 50 cases of the combination prolonged Q-T and cardiac syncopes have now been described

The first recognized case in Denmark is presented

The patient is a boy now aged 2 1/ year He is the first of two siblings born to health parents the hearing is normal there is n known familial deafness nor predisposition t epilepsy the pregnancy and delivery were nor mal and the infant did not have asphyxia

From the age of 14 months the boy had ha a total of ten attacks These commenced sud denly with violent screaming as if in pain Th hands were clenched and held in front of th

face The lips b came cyanotic and shortly afterwards h fell lost consciousness and had rattling respiration but no spasms After a few minutes he recovered consciousness and then slept for about half an hour after which h appeared completely normal again

The child was at first suspected of having epilepsy but all relevant investigations for epi lepsy had negative results

On the other hand after the attacks the electrocardiogram was abnormal with very varying T waves which gradually become nor malized while prolongation of the Q T interval persisted All electrolyte determinations showed normal findings Treatment with Digoxin was commenced The Q T interval returned to nor mal and the boy has been free from attacks for over 6 months

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Regional enteritis is a rare condition in child hood which is perhaps reported too seldom The symptoms are frequently generalized and

insidious Meticulous examination of extensive materials has revealed that, in 14% of the cases diagnosed in adult life, the first symptoms had occurred prior to the age of 15 years

Two cases are presented One was a boy aged 12 $^{3}/_{12}$ years On admission the symptoms had been present for 2 months in the *form of non specific systemic symptoms* anaemia, raised sedimentation rate and only slight and transient gastro intestinal symptoms The height was below normal and the state of nutrition so far below average that a malignant condition was immediately suspected The diagnosis was established by means of contrast enemata in the colon undertaken on account of slightly abnormal duodenal course observed following a barium meal and partly because of demonstration of port-wine discoloration of the urine on standing which proved to be due to indicanuria On laparotomy, severe changes in *the distal third of the ileum* the entire ascending colon and the greater part of the transverse colon *macroscopically and microscopically* compatible with Crohn s disease, were revealed Treatment in the form of resection of the colon with ileo sigmoidostomy and resection of the section of the ileum affected combined with postoperative medical therapy with diet and Salazopyrin resulted in complete recovery increase in weight and increase in height

The other case was a boy aged 13 years who was transferred from an ear nose and throat *department where he had been admitted in view* of tonsillectomy on account of tonsillar hypertrophy, anaemia and raised sedimentation rate but where he had developed diarrhoea Further investigation of the case history revealed periods with frequent loose motions during the preceding months On admission the patient was subfebrile The height was normal for the age but the state of nutrition was definitely below normal He did *not appear however* to suffer from chronic illness The subsequent course was *dominated* by intermittent fever the general condition deteriorated gradually and an abscess developed at the anus and proved difficult to treat Although radiographic examination of the alimentary canal including investigation of the appendix by Chrom s method and repeated barium enemata did not reveal any abnormalities apart from a para rectal abscess explorative laparotomy revealed pronounced changes in the distal third of the *ileum the* macroscopic appearance of which corresponded to Crohn s disease This diagnosis *could also be confirmed by biopsy from the* section of intestine involved As the remainder of the alimentary canal appeared to be entirely normal no resection was undertaken and instead the patient was treated postoperatively with diet and steroids and, later, with diet and Salazopyrin* which resulted in general recovery and thriving The subsequent course was complicated 6 months after the laparotomy by obstruction due to a band of adhesions which was treated surgically by intestinal resection On *this occasion, there was no evidence of active* entero colitis and since then the course has *been uncomplicated and favourable*

Neither of these patients presented any particularly alarming abdominal symptoms One of them had practically normal motions and both had normal or scarcely characteristic gastro intestinal passage on radiographic examination and nevertheless, they presented severe intestinal changes on explorative laparotomy The discoloration of the urine observed in the first patient, when *proved to be* due to indican suggested a pathological condition in *the small intestine* This does not appear to have been described previously although it is recognized that patients with this condition and a number of other conditions with increased bacterial growth in the small intestine and a number of conditions with malabsorption have excretion of indican in the urine Finally in the same patient it was observed that the Moro reaction became negative during the course of *the disease despite previous* BCG vaccination Similar observations have been made by other authors

N J Brandt

NEW BOOKS RECEIVED

J Huter & B Zehentbauer *Übergang von Medikamenten in Muttermilch und Nebenwirkungen beim gestillten Kind* 80 pp Georg Thieme Verlag Stuttgart 1970 DM 22 —

K Decker & H Backmund *Pädiatrische Neuroradiologie* 193 pp illus Georg Thieme Verlag Stuttgart 1970 DM 79 —

J L Melnick (ed) *Progress in Medical Virology* Vol 12 357 pp illus S Karger AG Basel Munchen Paris and New York 1970 sFr 72 — US $17.30

D R Laurence (ed) *Drugs Development and use* British Medical Bulletin Vol 26 1970 London £2

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F C Fraser & V A McKusick (eds) *Congenital Malformations* Proceedings 3rd International Conference on Congenital Malformations The Hague 1969 466 pp illus Excerpta Medica Amsterdam 1970 US $27 50

W Ludwig *Das Rechts Links Problem im Tierreich und beim Menschen* 496 pp illus Springer Verlag Berlin, Heidelberg and New York 1070 Price not given

G T Pack & A H Islami (eds) *Tumors of the Liver* Recent Results in Cancer Research Vol 26 304 pp illus Springer Verlag Berlin Heidelberg and New York 1970 DM 56 —

R W M Cammon *Human Growth and Development* 795 pp Charles C Thomas Publ Springfield Ill. 1970 US $9 00

Th. Ehrenpreis *Hirschsprung's disease* 175 pp illus Year Book Medical Publ Inc Chicago 1970 US $13 50

R E Gross *An atlas of children's surgery* 191 pp illus W B Saunders Co Ltd London 1970 £8 1s 6d

M Gunther *Infant feeding* 114 pp Methuen & Co Ltd London 1970 25s

R. R Rimner *Sex and the unborn child* 229 pp., The Julian Press Inc Publ New York 1969 US $6 95

Perinatal factors affecting human development Pan American Health Organization Scientific Publication No 185 Washington 1970

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G E W Wolstenholme & J Knight (eds) *Sensorial hearing loss* A Ciba Foundation Symposium 358 pp J & A Churchill Lt London 1970 80s

J M Berg B D McCreary M A C Ridler & G F Smith *The de Lange Syndrome* Institute for Research into Mental Retardation Monograph No 2 127 pp illus Pergamon Press Ltd Oxford 1970 63s

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BOOK REVIEWS

C C de Silva & N G Baptist *Tropical nutritional disorders of infants and children* Thomas Springfield Ill 1969 226 pp US $11 50

This book is a monograph of American Lectures in Living Chemistry The aim with this kind of book is to advance the newer knowledge of chemical medicine in the cause of clinical practice The interdependence of chemistry and medicine is so great that physicians are turning to chemistry and chemists to medicine in order to understand the underlying basis of life process in health and disease Of the authors Silva is the pediatrician and Baptist the biochemist and both are from Ceylon

The clinical and the biochemical aspects of the concept of growth and failure of growth are first discussed and the reader is given a few surveyable facts about several subjects The authors do usually not comment on these facts but they give many references for further studies Following the chapters on growth there are five chapters dealing with carbohydrates lipids proteins vitamins and minerals The emphasis is laid on the connection between these food factors and protein calorie malnutrition (PCM) The main part is about proteins and there is also a short survey of different individual amino acids in PCM One third of the whole book is about vitamines and the different deficiency syndromes The authors write very detailed and this reflects the importance of these diseases in India and Ceylon in contrast to many other tropical countries e g East Africa Vitamin B_{12} deficiency in infants probably caused by reduction of the vitamin in breastmilk is described The infants were very apathetic had a lemon yellow colour with symmetrical hyperpigmented arcinate patches They had involuntary movements of head trunk and limbs and the tongue was constantly protruded Arms and legs showed jerky movements and all had megaloblastic anemia The importance of giving potassium and magnesium in the treatment of PCM is stressed in the chapter about erythropoesis and anemia There are many references, but almost all are from years before 1967

For the pediatrician interested in nutrition his book gives a good introduction to the biochemical basis and the clinical interpretation of many common tropical disorders of infants and children

Bo Palldin

P F Bray *Neurology in Pediatrics* Year Book Medical Publishers Inc Chicago Ill 1969 514 pp illus. US $23 50

This textbook in pediatric neurology is divided in two parts the first dealing with differential diagnoses according to signs and symptoms, the second part giving more conventional description of various diseases One chapter describes the laboratory aids and their value in clinical work and another deals with practical problems in acute situations The tables are easy read and the illustrations instructive and mostly of good quality

Pediatric neurology is however a very vast subject and some parts of the book are rather short However a rather comprehensive literature list is given for the reader wanting further details In spite of being superficial in some topics the book gives a good survey of pediatric neurology It is quite useful as a reference book as well as for postgraduate studies

Ingrid Bjerre

ANNOUNCEMENT

The 1971 Annual Meetings of The American Pediatric Society Inc and The Society for Pediatric Research will be held at Traymore Hotel Atlantic City New Jersey

Wednesday April 28 8 30 p m *Does comprehensive care make a difference?* A symposium sponsored by the American Pediatric Society the Society for Pediatric Research and the Ambulatory Pediatric Society

Thursday April 29 *Plenary Session* American Pediatric Society

Friday April 30 *Subspecialties Session* The American Pediatric Society and The Society for Pediatric Research

Saturday May 1 *Plenary Session* Society for Pediatric Research

For information write to Charles D Cook M D (Secretary American Pediatric Society 333 Cedar Street New Haven Connecticut 06510) or Robert E Greenberg M D (Secretary Society for Pediatric Research 12012 Compton Avenue Los Angeles California 90059)

Acta Pædiat Scand 60 253–263 1971

IMMUNOFLUORESCENT AND MORPHOLOGICAL STUDIES IN CONGENITAL NEPHROTIC SYNDROME[1]

JUHANI RAPOLA and ERKKI SAVILAHTI

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The clinical course morphology and mode of inheritance of congenital nephrotic syndrome (CN) has been extensively studied and reviewed recently (8 22) The pathogenesis of the disease however has remained unsettled Kouvalainen (12) showed immunoglobulins attached to the glomeruli of 5 autopsy specimens and in one biopsy specimen using immunofluorescent techniques Similar results were obtained by Lange et al (14 15) and Kobayashi (11) in four additional cases These latter authors also found binding of components of complement in their studies

These results have been challenged by Hoyer et al (10) In studies of two monozygotic twins with characteristic CN they could not show IgG or β1C globulins in biopsy specimens taken at 6 weeks and 2 months of age respectively In the subsequent autopsy specimen of one of their patients at the age of 21 months they found slight to moderate staining for IgG and β1C proteins in most of the glomeruli in a local and uneven fashion They concluded that in their cases immune mechanisms are not primary in the pathogenesis of the disease

Recent attempts to improve the invariably fatal outcome of CN patients by kidney transplantation (9) has provided us ample material to reinvestigate the morphologic and possible immunopathologic changes in fresh CN kidneys

CASE REPORTS

Case 1

This boy was the first child in a family with no definite family history of congenital nephrosis However on the mother's side perinatal unexplained deaths had occurred in earlier generations The pregnancy was uneventful syphilitic serology during pregnancy was negative The child was born after 38 weeks gestation and his birth weight was 3 200 g and the weight of placenta was 1 070 g Because of the large placenta CN was suspected immediately after birth and heavy proteinuria (1 500 mg/100 ml) was noted At the age of 1 month the boy was transferred to our hospital At that time he was oedematous especially in the eyelids and the scrotum The abdomen was enlarged Urinary protein was 520 mg 100 ml Total serum protein was 3 1 g/100 ml Albumin was 0 4 g/100 ml α2 macroglobulins 1 57 g 100 ml and gammaglobulins 0 29 g/100 ml in cellulose acetate electrophoresis In immunoelectrophoresis the same changes were noted and in addition elevated IgM was seen Serum cholesterol and triglycerides were elevated 374 mg/100 ml and 11 5 mM l respectively Angiography of the inferior cava showed it to be patent

Nephrectomy was performed at the age of 3 months in order to perform kidney transplantation

Case 2

This boy was the second child of parents with no family history of CN Their first child was in good health Pregnancy was normal and serological tests for syphilis during pregnancy were negative The child was born after 37 weeks gestation his birth weight was 2 650 g and the weight of placenta was 1 000 g He was admitted to our hospital at the age of 2 weeks because of cyanosis and vomiting At that time he was oedematous especially in the eye

[1] This study is published in honor of Professor Dr med K H Schafer Hamburg at his 60th birthday

lids The abdomen was enlarged but no ascites was seen on an X ray Urinary protein was 210 mg/100 ml Serum protein concentration was 3 9 g/100 ml In cellulose acetate membrane electrophoresis al bumin was 0 5 g/100 ml α2 macroglobulins 2 1 g/100 ml and gammaglobulins 0 1 g/100 ml The same changes were seen in immunoelectrophoresis in ad dition elevated IgM was seen and it was quantita tively 170 mg/100 ml Cholesterol was 850 mg/100 ml At the age of 6 months angiography of the in ferior cava showed it to be patent He was treated several times in hospital and nephrectomy was done at the age of 1 year 11 months in preparation for kidney transplantation

MATERIALS AND METHODS

Whole kidneys were obtained immediately after sur gical nephrectomy Pieces of the tissue for light microscopy electron microscopy and immunofluores cent microscopy were processed immediately and the remainder was frozen and stored at −20 C

Light microscopy Several 3 mm thick pieces of tissue consisting of both cortex and medulla were fixed in Bouin s fluid and paraffin sections of 2-3 μm were stained with hematoxylin and eosin van Gieson s PAS Mallory s trichrome and Gomori s periodic acid silver methenamine

Electron microscopy Several 1 mm pieces of cor tical tissue were fixed in 2 5 phosphate buffered glutaraldehyde at pH 6 8 postfixed in 1 phosphate buffered osmium tetroxide at pH 6 8 dehydrated and embedded in Epon 812 1 μm plastic sections were stained with 1° aqueous toluidine blue for orienta tion Thin sections were stained with uranyl acetate and lead citrate Three mature glomeruli from both cases were studied in a Zeiss EM 9A electron micro scope

Fluorescent microscopy Commercial fluorescein isothiocyanate (FITC) labelled goat antisera to human IgG IgM and IgA (Hyland laboratories inc Calif USA) were used Rabbit antiserum to human β1C globulin was prepared according to Stratton (24) and labelled with FITC (4 18) Fluorescein/protein ratio was 2 7 (26) In blocking controls commercial unlabelled goat antisera to human IgG (Mann re search laboratories inc N Y USA) IgM (Mann) and IgA (Hyland) were used and the unlabelled antiserum to β1C globulin was the same as that used for preparation of the FITC conjugate Mono-specificity of the antisera was checked by immuno electrophoresis and double diffusion micromethod (3)

For direct immunofluorescent study small pieces of kidneys consisting both of cortical and medullary tissue were frozen with solid carbon dioxide Sec tions of 5 μm were cut in a cryostat microtome at −20 C picked up on microscope slides and dried at room temperature for 30 min All subsequent pro cedures were performed at room temperature Sec tions were washed for 4 hours in phosphate buffered isotonic saline pH 7 2 (PBS) fixed in ethanol me thanol and chloroform (1 1 1 mixture) for 30 min and washed in three changes of PBS 10 min in each Excess PBS was drained off and the sections were overlayed with FITC conjugates They were incubated in a humid box for 1 hour and washed in three changes of PBS 10 min each The slides were swept dry around the sections mounted in PBS buffered glycerol (1 10) under coverslips and ex amined immediately

HB 200 mercury vapour lamp was used as a light source UG 5/1 mm and BG 12/1 mm excitation filters and a K 460 blocking filter were used for examination and photography in colour on Kodak Ektachrome daylight type film For black and white photography Kodak Tri X film was used together with a BG 12/5 mm excitation filter in combination with a K 530 blocking filter A Leitz Ortholux micro-scope with cardioid dark field condensers was em ployed in these studies

FITC conjugated antisera were diluted to a point giving bright specific fluorescence with minimal back ground on kidney biop s taken from various glo merulonephritic disorders and processed similarly In these previous studies a distinct linear or granular fluorescence along the glomerular capillary base ment membrane was discerned depending on the character of the disorder The specificity of staining was controlled by blocking procedures with un labelled antisera causing clear diminution of the staining

Kidney for indirect immunofluorescent study was obtained at surgical biopsy It proved to be morpho logically normal and negative on direct immunofluo rescent studies Tissue sections were fixed either in ether alcohol for 10 min each (16) or processed as in direct immunofluorescent staining When the ex cess PBS was wiped off the sections were covered with eluate from nephrotic kidneys (see following paragraph) Slides were incubated in a humid box for one hour washed in two changes of PBS for 10 min wiped dry and covered with FITC con jugates Antihuman IgG IgM and IgA FITC con jugates were used in serial dilutions (1 2-1 32) Af ter incubation for 1 hour sections were washed and processed as described before A similar eluate from a normal kidney obtained at autopsy and serum IgG (1) served as a control

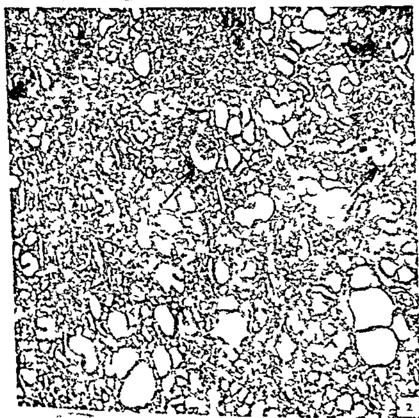
Elution experiments Half of one kidney of both cases and half of a morphologically normal control kidney obtained at autopsy 12 hours after death were processed according to the method of Lerner et al (16) with slight modifications

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Fig 1 Kidney cortex from the case 1 Most glo-meruli at the low magnification are relatively nor mal and dilatation of tubuli is mild and patchy H & E ×60

Fig 2 Kidney from case 2 Glomeruli with con densed capillary tuft and widened urinary space (ar rows) are common Extensive tubular dilatation with some tubules containing casts is prominent H & E ×60


1


2

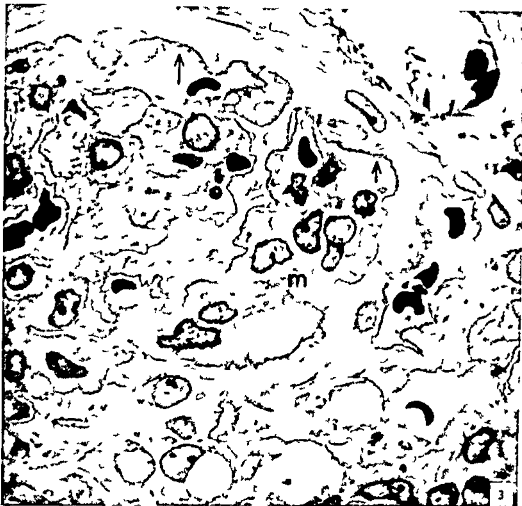


Fig 3 Portion of a glomerulus from case 2 Widening of the mesangial area (*m*) with hyperplasia of mesangial cells is evident Peripheral capillary loops are widely open and the basement membranes (*arrows*) thin Epon Toluidine blue ×1 400

The fragmented kidney pieces were washed for a short time and homogenized with Ultra Turrax for six times 5 sec at 0 C Homogenates were suspended in PBS pH 7 2 and centrifuged at 23 000 g for 10 min at +4 C Washing was repeated 10 times with PBS After washing the homogenate was eluted with 0 02 M citrate buffer pH 3 2 (10 ml of buffer for 1 g of kidney tissue) at 37 C under constant stirring for 2 hours and centrifuged at room temperature at 23 000 g for 10 min Supernatant was neutralized with NaOH 0 1 N to pH 7 and dialysed to PBS The protein concentration was determined and the eluate was concentrated by negative pressure in Visking dialysis bags Concentrates with a protein concentration of about 60 mg/100 ml and 500 mg 100 ml were used for indirect fluorescent studies Concentrates were analysed using cellulose acetate membrane electrophoresis immunoelectrophoresis with anti human whole serum single radial immuno diffusion (19) and double diffusion micromethod (3) Antisera to human IgG IgA IgM and β1C globulin were used in these determination The sensitivity of double diffusion technique used for serum immuno globulins is 0 5 mg/100 ml

RESULTS

Light microscopy The histological picture of both cases differed markedly (Figs 1 and 2) In case 1 the changes were slight but distinct Most glomeruli showed slight to moderate mesangial cell proliferation with increase of PAS and silver positive fibrillar material in mesangial areas (Fig 3) Thickening or splitting of peripheral capillary basement membranes was rarely found In some glomeruli the urinary space was widened and occasional foetal microglomeruli were seen Cystic dilatation of proximal tubules was rare Hyaline casts were seen in some dilated proximal and distal tubules No interstitial fibrosis nor accumulations of inflammatory cells were present

In case 2 the pathological changes were



Fig 4 Electron microscopical picture of a glomerulus from case 1 There are hyperplastic mesangial areas. Collections of tortuous basement membrane like material are conspicuous Epithelial foot processes have disappeared and numerous epithelial microvilli are present Hypertrophic endothelial cytoplasm is seen in some capillary loops and bulging of mesangial cytoplasm into a capillary loop is seen The basement membrane itself is thin. *M* Mesangial area *m* mesangial cytoplasm bulging into the capillary loop *V* epithelial microvilli *E* endothelial cell with abundant cytoplasm *C* capillary lumen × 3 250

more severe Approximately half of the glomeruli showed mesangial reaction as in the previous case The rest of the glomeruli were in various stages of hyalinization and fibrosis and showed proliferation of several types of glomerular cells In several glomeruli cystic dilatation of the urinary space and atrophy of the glomerular tuft was seen (Fig 2) Ex

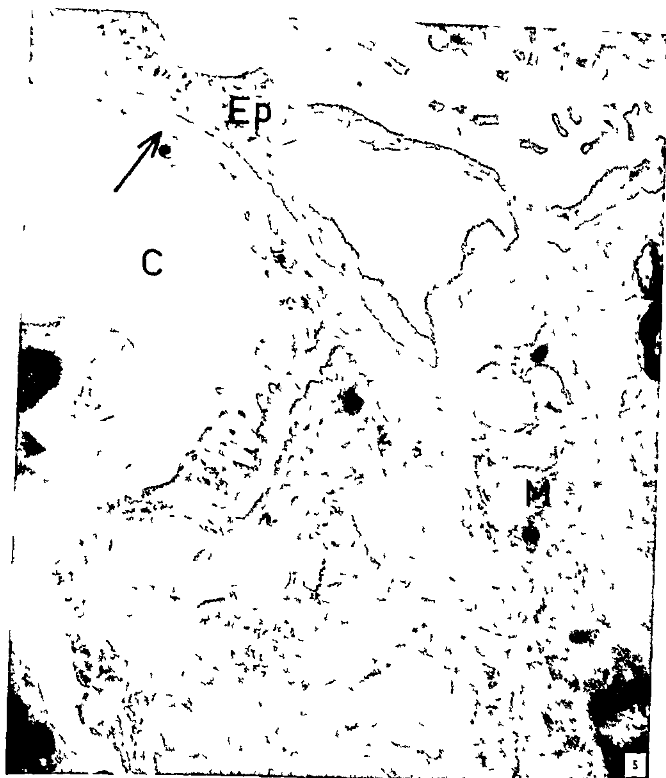


Fig. 5. Detail of a capillary mesangial junction. Tortuous material with a density like that of basement membrane is accumulated in mesangial area and it is partly projecting in the capillary space. Foot processes have disappeared from the epithelial covering of the basement membrane. *M*, mesangial area; *C*, capillary lumen; *Ep*, epithelial cytoplasm; arrow shows basement membrane. ×17 500.

tensive microcystic dilatations of both proximal and distal tubules governed the picture. The dilated tubules contained numerous hyaline casts. Atrophic tubules were dispersed between the cystic areas. There was prominent diffuse interstitial fibrosis and accumulations of inflammatory cells mainly close to the capsule.

Electron microscopy Three mature looking glomeruli from each case were studied Fusion of the epithelial foot processes and numerous microvillous projections of the epithelial cells were a common feature in both cases (Fig 4) As could be predicted from the light microscopy mesangial areas were widened and showed tortuous matrix with staining charac teristics identical with the basement mem brane (Fig 5) No appreciable granular de posits were found The number of mesangial cells was clearly increased The basement membrane was thin in general but in some place its borders on both epithelial and endothelial side were slightly uneven and there was some thickening and mottling of the lamina densa No electron dense deposits were associated with the basement membrane Oc casional obstruction of capillary loops was caused by increase of endothelial cytoplasm containing well formed organelles and nu merous microvesicles To this was added protrusion of mesangial cytoplasm into the capil lary lumen (Fig 4)

Fluorescence microscopy No glomeruli showed fluorescence with any of the antisera used Positive staining however was found in dense casts of tubular lumens and quite frequently also inside the tubular cytoplasm (Figs 6 and 7) The tubular casts were stained most frequently by anti β1C-antiserum al most as frequently with anti IgG serum less often with anti IgM serum and only occasion ally with anti IgA serum Cytoplasmic stain ing could be shown with anti β1C IgG and IgM sera but not with anti IgA serum

Elution experiments The wet weight of the tissue to be eluted was 7 4 g in case 1 9 0 g in case 2 and 13 0 g in control case Citrate buffer eluate contained 3 6 mg 2 5 mg and 2 7 mg of protein per 1 g of tissue respec tively Protein concentrations of the eluates for the following analysis were 460 mg/100 ml 470 mg 100 ml and 540 mg/100 ml respec tively Electrophoresis on cellulose acetate membrane showed protein in the gamma globulin region only in eluates of nephrotic kidneys In the eluate of control kidney there was in addition to gammagloublin a protein in the β region On double diffusion plates only IgG could be detected in all three eluates The IgG concentration in case 2 and the control was about 5 mg/100 ml and in case 1 above 2 mg/ml but could not be exactly quantitated The eluated IgG calculated from these values is approximately 15–35 μg per gram of kidney tissue and no difference be tween the amounts of nephrotic kidneys and the control was apparent

When kidney sections of a normal surgical biopsy were incubated with the eluates in two different concentrations (60 mg/100 ml and concentrations as stated above) and subsequently stained with FITC conjugated anti sera no specific staining of glomerular or tubular components was found

DISCUSSION

We cannot offer reasonable explanation for differences in the immunofluorescence stud ies on kidneys of CN children presented by various authors It is possible that all nephrotic syndromes beginning in early infancy do not belong to the same aetiological group By reviewing the published results this seems not to be the explanation of some of the con tradictory results All cases of Kouvalainen (12) and those of Hoyer et al (10) and our were of Finnish extraction suggesting a com mon genetic origin (22) As far as one can judge from the published reports the clinical course and kidney morphology was very much alike in these cases It is possible that at the terminal phase of the disease new additional pathogenetic mechanisms can appear with con comitant immunologic injury Five out of the 6 cases presented by Kouvalainen were au topsy studies and in the kidney obtained at autopsy by Hoyer et al some immunoglobulin staining in the glomeruli was demonstrated Our first case represents a morphologically rather early phase of the disease while the second case is an advanced one The tissue

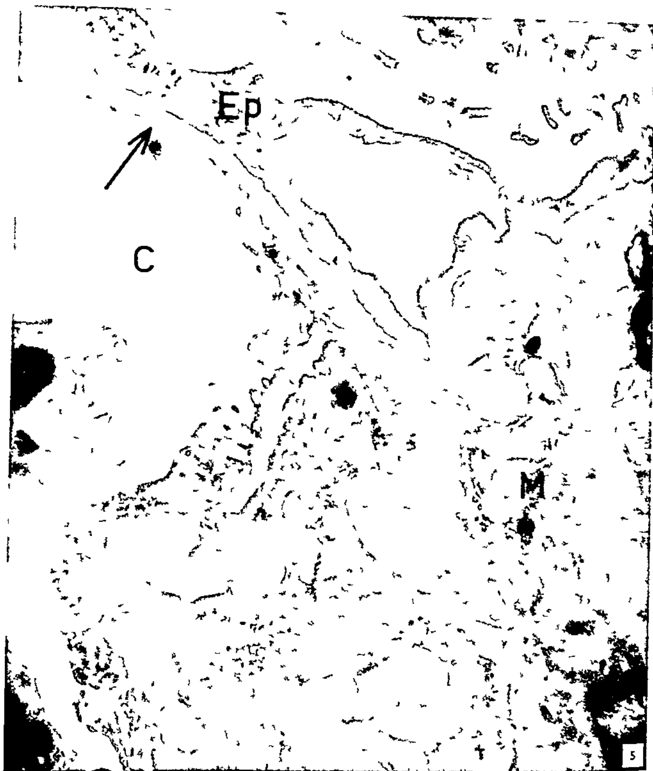


Fig 5 Detail of a capillary mesangial junction Tortuous material with a density like that of basement membrane is accumulated in mesangial area and it is partly projecting in the capillary space Foot processes have disappeared from the epithelial covering of the basement membrane *M* mesangial area *C* capillary lumen *Ep* epithelial cytoplasm arrow shows basement membrane × 17 500

tensive microcystic dilatations of both proximal and distal tubules governed the picture The dilated tubules contained numerous hyaline casts Atrophic tubules were dispersed between the cystic areas There was prominent diffuse interstitial fibrosis and accumulations of inflammatory cells mainly close to the capsule

however was obtained fresh and may thus be better suited for this type of study than tissue obtained at autopsy

It is generally known that immunofluorescent techniques even to day are capricious Tissue handling purity specific antibody titer and labelling of the antiglobulins optical system and finally interpretation of the staining may affect the results This is especially true when the amount of antigenic material is present in small quantities In addition to the usual control procedures we have further tested the monospecificity of our antihuman immunoglobulin antisera and their ability to stain intracellular immunoglobulins and the lack of crossreactivity with gastrointestinal specimens (23)

Electron microscopy gave further support to the absence of immunocomplexes in the glomeruli We did not see clear nodular deposits on the epithelial side of the basement membrane as is seen in poststreptococcal glomerulonephritis (21) or on the endothelial aspect as occurs in lupus erythematosus (7) Thickening of the capillary basal membrane as seen in case 2 was focal and local and did not correspond to the changes in the full blown glomerulonephritides caused by anti basement membrane antibodies as in Good pasture s syndrome (5) Enlarged mesangial areas showed increased matrix (basement membrane like material) but no granular electron dense deposits in any appreciable amount as in the anaphylactoid purpura with kidney involvement (25) Close correlation between electron dense deposits and immunoglobulin binding has been observed in these disorders

In recent studies it has been possible to elute and characterize antibodies from immunocomplexes in kidneys with the antibasement membrane type of glomerulonephritis (16 20) lupus nephritis (13) and experimental streptococcal glomerulonephritis (17) Although our immunofluorescent studies appear to make it unlikely that immunocomplexes exist in glomeruli of our patients we wanted to check this with elution experiments An additional reason for elution experiments was the unexplained fluorescent staining of the tubular epithelium. This staining combined with the accumulation of lymphoid cells between the tubules led us to suspect the possibility that autoimmune mechanisms might exist as a consequence of the tubular destruction The fluorescence would thus represent antibodies directed toward autologous tubular antigens If this were true it could later result in an immunocomplex type of glomerulonephritis as occurs in the experimental allergic glomerulonephritis caused by antitubular antigens (6) The acid eluate contained a minimal amount of antigenic IgG which was also recovered from the control kidney Lerner et al (16) eluted about 100 μg of IgG per gram from a glomerulonephritic kidney with Goodpasture s syndrome Our experiment showed only $^1/_3$ or less IgG of that value The indirect immunofluorescent staining with eluted protein did not support our hypothesis of antitubular antibodies For firm conclusions however more controlled experiments are required to prove or disprove this hypothesis Unfortunately no material with established immunocomplex disease was available to control the effectivity of the elution experiments

Our present immunofluorescent studies supported with electron microscopic findings did not disclose any evidence for the presence of immunological mechanisms in the pathogenesis of the congenital nephrotic syndrome in accordance of the earlier results of Hoyer et al (10)

SUMMARY

Two kidneys from patients with congenital nephrotic syndrome obtained at nephrectomy at the age of 3 and 23 months were investigated for possible immunopathological mechanisms for the kidney injury Immunofluorescent staining with anti IgG IgM IgA and β1C globulins did not show attachment of immunolglobulins or complement to the

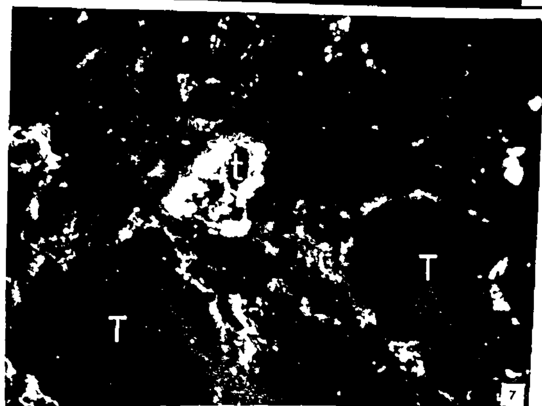


Fig. 6. Kidney section of case 2 stained with anti-IgG. Casts in the dilated tubules show positive fluorescence as the glomerulus (*G*) is quite negative. ×300.

Fig. 7. Kidney section of case 2 stained with anti-β_{1C} globulin. Faint staining of the epithelium of dilated tubules (*T*) is visible. Bright intraepithelial staining of a small tubule (*t*) is evident. ×300.

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glomeruli Staining was found in some tubular casts and occasionally in tubular epithelium as well Electron microscopic studies of the glomeruli corroborated the idea that immunocomplexes are not conglomerated in the glomeruli because of the absence of typical deposits which are usually found in association with positive immunofluorescent staining in various glomerulonephritides

Kidneys were also eluted with acidic buffer in order to remove antibodies from immuno complexes The eluates were analyzed for their immunoglobulin content and used for indirect immunofluorescent staining The content of IgG of the kidney eluates was low and no difference of eluted IgG between the nephrotic kidneys and the control was apparent No binding of immunoglobulin with structures of normal kidney could be seen in the indirect immunofluorescent study

Our results corroborate the previous results (10) that immunopathogenesis is not essential in the congenital nephrotic syndrome

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Key words Congenital nephrotic syndrome immunofluorescence electron microscopy

glomeruli Staining was found in some tubular casts and occasionally in tubular epithelium, as well Electron microscopic studies of the glomeruli corroborated the idea that immunocomplexes are not conglomerated in the glomeruli because of the absence of typical deposits, which are usually found in association with positive immunofluorescent staining in various glomerulonephritides

Kidneys were also eluted with acidic buffer in order to remove antibodies from immuno complexes The eluates were analyzed for their immunoglobulin content and used for indirect immunofluorescent staining The content of IgG of the kidney eluates was low and no difference of eluted IgG between the nephrotic kidneys and the control was apparent No binding of immunoglobulin with structures of normal kidney could be seen in the indirect immunofluorescent study

Our results corroborate the previous results (10) that immunopathogenesis is not essential in the congenital nephrotic syndrome

ACKNOWLEDGEMENT

We are indepted to the kidney transplantation team (9) for providing the material for this study The antiserum to β1C globulin was a gift from Dr E Jokinen Cellulose acetate membrane electrophoresis was kindly performed by Miss E L Rahiala M Sc

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HYPOSENSITIZATION IN HOUSE DUST ALLERGY ASTHMA

A Double blind Controlled Study with Evaluation of the Effect on Bronchial Sensitivity to House Dust

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A placebo controlled study of the effect of hyposensitization was performed in 93 children shown to suffer from asthma caused by bronchial allergy to house dust The allergy diagnosis was substantiated by bronchial provocation tests (BPT) and the effect of the injection treatment was assessed by estimation of the degree of bronchial sensitivity to house dust before and after treatment (2) The specific hyposensitization was found to be superior to placebo injections as concerned improvement of allergic responsiveness of the bronchi The data relevant to the study of the effect on bronchial sensitivity and subjective improvement are presented in this paper

MATERIAL AND METHODS

Th patients were selected among children 2–14 years old who had been referred for bronchial asthma Only those who had such severe asthma that active treatment with hyposensitization seemed justifiable were included in the study Additional requirements were that the patients reacted with bronchial obstruction to bronchial provocative tests with both of the two house dust extracts used in this study and that they had not received hyposensitization with house dust extracts prior to the study

The same allergen extracts were used both for skin testing bronchial provocative tests and hyposensitization Two house dust extracts were purchased from Nyegaard et Co Oslo (house dust A) and from Allergologisk Laboratorium Copenhagen (House dust B) respectively Saline (0 9 per cent) containing 0 5 per cent phenol was used as placebo and a brown sugar stain was added to make the placebo lo similar to the house dust extracts Both extracts a placebo were similarly bottled in randomly cod vials by Nyegaard et Co and the top concentratio was indicated as 1 10 The code was broken aft the final evaluation of the effect of treatment.

The diagnostic procedures used for the selection patients were as described in previous papers (1 The bronchial provocation tests were initiated at lo concentrations of the allergen extract and the concentration was then gradually increased The minimal dose needed to elicit significant bronchial obstruction was recorded as the particular bronchial sensitivity threshold dose (2)

The hyposensitization treatment was undertaken by the local physician who had referred the patient. Using this approach it was assured that local reactions elicited by the extract injections could not be registered by the persons who finally evaluated the treatment The injection treatment was started with the extract or placebo diluted with saline containing 0 5 phenol to a concentration of 1 1 million Injections were given 1–2 times weekly until 0 5–0 9 ml of the top concentration was reached If reactions to the injection occurred the physician was in tructed to reduce the dose and continue with the highest tolerated dose for maintenance The maintenance dose was given with 2 to 4 week intervals and this was continued for 2 ½–3 years until the re-evaluation of the bronchial sensitivity

The effect of the treatment was evaluated by means of repeated bronchial provocative tests with estimation of the specific bronchial sensitivity threshold to the house dust extracts When no bronchial reaction could be provoked by the inhalation of 1 ml of the top concentration (1 10) of the extract the improvement of bronchial tolerance was registered as 100 per cent Partial improvement was recorded accordingly The actual increase of the bronchial threshold dose was expressed as the p rcentage of the increment between th starting threshold dose and

Table 1 *Data for patients omitted from the study*

Patient code	Reason for omission	Treatment group	Improvement: Subjective	Improvement: Bronchial
9	Too much asthma Corticosteroids	A	O Corticosteroid dependent	Not assessed
13	Stopped because of local and eczematous reactions after 10 months treatment	A	Much improved	100 ~
27	Too much asthma	A	No change	0
49	Switched to know genuine extract	A	Symptom free	100
74	BPT not performed after treatment	A	Much improved	Not assessed
14	Stopped because physician does not believe in the treatment	B	Some improvement	0
41	Injections too irregularly given	B	Much improved	75
61	Too much asthma Corticosteroids	B	Cortisteroid dependent	Not assessed
35	Switched to known genuine extract	C	Much improved	100 ~
51	BPT not performed after treatment	C	Much improved	Not assessed
66	Switched to known genuine extract	C	Much improved	Not assessed
94	Injections not given as instructed	C	Much improved	0
96	Stopped because physician does not believe in the treatment	C	No change	0

1 ml of the top concentration For this approximation this increment was plotted on a linear scale each 1 10 dilution represented by identical values. Care was taken to p rform the bronchial provocation tests under conditions comparable to those of the initial ones (?)

Data were collected also for assessment of the g eral clinical condition before during and after the injection treatment These comprised symptom diaries, records of medication used days lost from school and the parents impression of the degree of improvement durin the house dust season (October–March) In the present report weight is given only to the effect on the bronchial sensitivity to house dust, but this effect will be compared with the subj ctive impression of the parents

RESULTS

Thirteen of the 93 patients originally admitted to the study had to be omitted for reasons summarized in Table 1 Five of the omitted patients belonged to the group House dust A" 3 to the group "House dust B and 5 to the group receiving placebo injections The bronchial sensitivity and subjective improve m nt were assessed in 8 of the 13 patients omitted (Table 1)

The study was completed for 80 patients Hou e dust A had been given to 31 house dust B to 21 and placebo to 28 patients respectively (Fig 1) In the house dust A and house dust B treated groups complete bronchial tolerance to the house dust provocations had been achieved in 21 and 14 patients respectively compared with only 7 in the placebo group When those with marked improvement of bronchial tolerance (more than 75 per cent of maximum possible improvement) were included the figures were 26/31 19/21 and 9/28 respectively—showing that both house dust extracts were significantly superior to the placebo ($p < 0.01$)

Changes of bronchial sensitivity before and after the injection treatment were compared with the clinical improvement as subjectively judged by the parents (Fig 2) Mo t of the house dust treated patients showed marked improvement and most of the placebo-treated patients did not with respect to both parameters but full correlation was not observed By subjective measures 2 patients were classified as worse or unchanged although full tolerance to the house dust bronchial provocation had been achieved and 6 patients were classified as much better although the bronchial sensitivity to house dust appeared to be unchanged or even worse

DISCUSSION

Bronchial allergy is a frequent cause of asthma (1 2) Several allergens precipitate attacks of

bronchial obstruction in sensitized individuals, and house dust allergens are common offenders in this respect (2 18) Many patients are allergic to more than one allergen, however, and even in extrinsic asthma various non allergic factors contribute to the disease both as concerns acute attacks and the long term effects (1)

As could be anticipated, complete correlation between immunological treatment with one allergen type and clinical improvement was not achieved Both bronchial hypersensitivity and general clinical improvement are subject to other influences than those effected by immunotherapy Bronchial allergy is influenced by the degree and nature of exposure as well as factors determining the general and specific reactivity of the organ in question This necessitates the use of placebo controlled systems

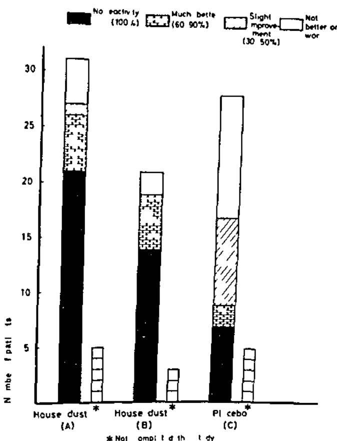


Fig 1 Effect of therapy on bronchial hypersensitivity to house dust Degree of improvement as assessed by bronchial threshold dose for allergen before and at completion of study Results of control provocation after 2 $^1/_2$–3 years treatment *Ordinate* number of patients in each group

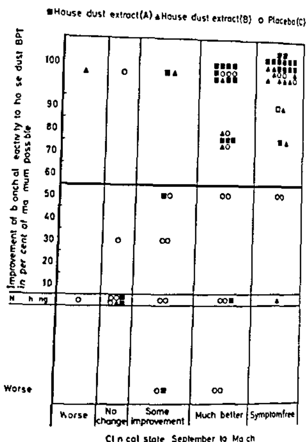


Fig 2 The improvement as assessed by bronchial sensitivity threshold dose before and at completion of study (*ordinate*) compared with the subjective improvement as judged by the impression of the parents (*abscissa*) *BPT* Bronchial Provocation Test

when the effect of a particular treatment is to be evaluated

In the treatment of bronchial asthma, patients receiving placebo instead of allergen injections regularly show some improvement with a placebo effect similar to that noted in attempts to evaluate therapy in such diseases as angina pectoris, the common cold, and diabetes mellitus (3, 4 6 11, 12, 13, 15) This is not necessarily due to beneficial psychosomatic effects of the placebo The injection treatment, be it with extract or placebo is in fact only an adjuvant to several other therapeutic measures, and the injections may be in different components in an otherwise effective multi potent treatment The treatment also involves various psychosomatic mechanisms including the doctors influence on the patients attitude to the disease Parameters for evaluation of the treatment such as days lost from

school or work reduction of symptomatic medication and subjective well being are as much indicators of changes of the patients attitude to the disease and effectiveness of simultaneous therapy as they are of benefits of the injection treatment on the bronchial reactivity as such

Even when placebo-controlled studies are performed errors may arise leading to false conclusions Assessment of therapeutic effect is not possible if the diagnosis is inconclusive or incorrect Other critical points to be considered are selection of patients diagnostic criteria quality of allergen extract and placebo optimal use of the therapeutic measure to be studied and parameters for the evaluation of the effect (12 13) Several double blind controlled studies suggest that specific hyposensitization is superior to placebo injections in the treatment of allergic diseases (4 6 7 11 12 16) However a few negative reports have appeared Fontana et al (3) conducted a double-blind study in children with ragweed hay fever The parents kept a symptom record which was finally scored for the presence or absence of symptoms whereas the severity and duration of symptoms were not noted There was no difference between the treated group and the placebo group in this study Critical objections are that the treatment apparently was too short and the type of evaluation used did not detect any amelioration less than 100 per cent One report presented by the Research Committee of the British Tuberculosis Association (BTA) suggested that hyposensitization with house dust was ineffective in bronchial asthma (15) However this study did not fulfill the demands of a scientific approach to treatment evaluation (5) The diagnosis of bronchial allergy to house dust was made by history and skin tests only The specific allergy diagnosis may be subject to a 20-50 per cent methodological error when based on such criteria only (1 2) Albeit previous studies have repeatedly shown that hyposensitization should be carried out with a maximum tolerated dose and for a rather prolonged time (4 6 7 11 12 16) the treatment in the BTA study consisted of a rather short course of injections following either a routine non individualized set up or a course which was brought to an end if the patient showed untoward reactions The evaluation was based on the patients subjective impression Obviously this sort of study is of restricted value

The results of several other controlled studies suggest that hyposensitization with selected allergens is effective and specific provided the doses are large enough and that the treatment is prolonged (4 6 7 11 12 13 14) This has also been substantiated by experimental studies with sensitized animals and sensitized cells in cell suspensions The latter studies have shown that hyposensitization has positive effects also at the cellular level although the basic mechanisms at work are not clarified (8 9 10 14 16 17 19)

The lack of controlled information about the effect of hyposensitization on the bronchial hypersensitivity to the particular allergen has been a kind of missing link in the chain of evidence The results of the present study appear to consolidate this chain

SUMMARY

A double blind controlled study of the effect of house dust hyposensitization was conducted in 93 patients allergic to house dust. The allergy diagnosis was established by bronchial provocation tests and the degree of bronchial sensitivity to house dust extracts (bronchial threshold dose for allergen) was assessed before and after 2 1/2-3 years injection treatment

The specific hyposensitization with two different house dust extracts was superior to placebo injections

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I gratefully acknowledge the assistance of Nyegaard and Company in providing extracts in randomly coded vials and my thanks are also due to nurses Anne Kasin and Valborg Lachmann Mørck for skilled technical assistance

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PROTHROMBIN IN NEWBORNS AND DURING THE FIRST YEAR OF LIFE

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The hypoprothrombinemia of the newborn was first demonstrated by Brinkhous et al in 1937 by use of their two-stage prothrombin assay (5) Waddell and coworkers (41) were the first to associate the hypoprothrombinemia expressed by a prolonged "prothrombin time (34) with vitamin K deficiency Several investigators (7 29 30 35) reported that prothrombin decreased during the first few days of life and then increased to normal values within a week (35) or within a year (5 6 30) depending on the method used for the assays It has been postulated that the postnatal decrease of prothrombin and related coagulation factors might be explained by a quick reduction of the transferred maternal factors combined with a slow synthesis of coagulation factors in the infant liver (16) Because of the potential bleeding risk in this age period vitamin K prophylaxis was introduced and a safe dosage established (1 6 10 36)

Studies of prothrombin and other coagulation factors in the newborn have resulted in some paradoxical findings Thus the one stage prothrombin time according to Quick (34) gives normal values in newborns in spite of definitely subnormal activities of factors VII X and prothrombin (factor II) assayed by specific methods In adults the Quick test registers deficiencies of these factors Several ex[illegible] nolecular differences between infant and adult prothrombin (30 36) and fibrinogen (20 42) or possibly an excess of factor V (11) in the newborn It is evident that testing techniques used must always be taken into account when comparing coagulation assay results especially in this age group (1)

In most Swedish hospitals "prothrombin index according to Quick Lehmann (23 34) and the prothrombin proconvertin assay according to Owren & Aas (31) have been replaced by thrombotest according to Owren (32) as the latter test is performed with a ready-made freeze dried reagent and is well standardized The Quick Lehmann test registers deficiencies of factors II V VII X as well as fibrinogen (factor I) but with varying sensitivity The prothrombin proconvertin and the thrombotest reagents contain bovine fibrinogen and factor V and the methods register deficiencies of vitamin K dependent coagulation factors i e factors II VII and X and the thrombotest to a certain extent also factor IX

The present investigation was undertaken to study a new specific two-stage assay of prothrombin (3 28) in newborns Further the correlation between thrombotest activity and prothrombin concentration was studied as well as prothrombin and proconvertin during the first year of life The study was especially concerned with cases of decreased prothrombin

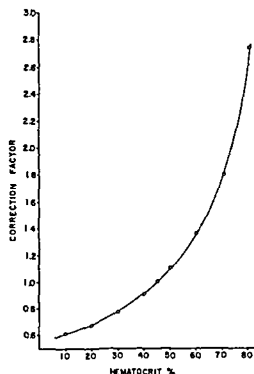


Fig 1 Correction for hematocrit was made by a correction factor calculated according to the expression $F = (100 - Hn)/(100 - H)$ *Hn* normal mean value for hematocrit (45 % used here) *H* hematocrit of sample Correction factor (*F*) was plotted against hematocrit (*H*)

content, aiming at establishing a borderline value beneath which treatment with additional vitamin K or plasma should be given to prevent bleeding

METHODS

Thrombotest was performed by addition of 0 05 ml capillary blood mixed with 0 05 ml citrated saline to 0 25 ml of reconstituted warmed reagent (37 C) and measuring the clotting time (19)

Table 1 *Prothrombin determinations (28) in mixtures of citrated plasma and washed red cells*

Hematocrit of micture ( )	Correction factor for hematocrit	Obtained prothrombin value	Corrected prothrombin value
20	0 68	146	102
30	0 78	128	102
40	0 91	108	100
45	1 00	100	100
50	1 10	92	101
60	1 37	72	99
70	1 80	54	98
80	2 75	36	99

Prothrombin was determined with a two-stage assay using a freeze-dried reagent of intrinsic coagulation factors (28) In this method 0 025 ml capillary blood diluted 1 3 with citrated saline is added to 1 ml of reconstituted warmed (37 C) reagent After a certain incubation time 0 2 ml of this mixture is added to 0 2 ml of warmed (37 C) fibrinogen solution and the clotting time determined by aid of a coagulometer (Depex de Bilt Holland)

Prothrombin and proconvertin (P P) was determined in capillary blood according to Sundblad's modification of Owren s method (31 38) In this modified method capillary blood is diluted with buffered citrated saline The blood is centrifuged and 0 2 ml of the supernatant is added to 0 4 ml of reagents The mixture is recalcified and the clotting time determined

Hematocrit was determined in capillary blood with heparinized microhematocrit tubes inner diameter 0 5–0 6 mm length 75 mm The tubes were centrifuged for 3 min at 11 000 r p m in a microcapillary centrifuge (International Equipment Company Boston USA)

Correction for the hematocrit of the blood sample was performed by aid of the curve shown in Fig 1 The validity of this correction was tested on mixtures of citrated plasma and washed red blood cells (Table 1)

MATERIAL

The *clinical material* included 100 infants aged 0 ? days from the Department of Pediatrics Karolinska sjukhuset Stockholm hospitalized under various diagnoses hyperbilirubinemia 20 asphyxia 25 respiratory distress syndrom (RDS) 11 immaturity 10 dysmaturity 4 infections 7 VOC 5 other diagnoses 1 Moreover 61 healthy full term newborns aged 0-? hours from the Department of Obstetrics at the Karolinska Sjukhuset Stockholm were investigated as well as 98 healthy children aged 0–12 months from nursery homes in Gothenburg All newborns in nursery given 1 mg vitamin K (Konakion®) intramuscular immediately after birth In some asphyctic newborns vitamin K was given somewhat later when other vitally indicated treatment had been inserted No vitamin K was given to the mothers

The investigation was with short interruptions continued over a period of one year Samples were taken only on week days during day time

RESULTS

Correlation between prothrombin and thrombotest values

In Fig 2 is shown the correlation between prothrombin and thrombotest values for the entire clinical material with and without correction for hematocrit As can be seen there is a te

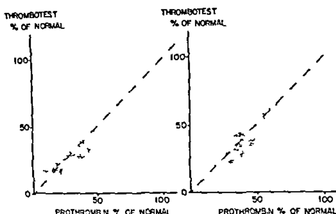


Fig 2 Prothrombin and thrombotest values of the clinical material from Karolinska Sjukhuset I values not corrected for hematocrit II values corrected for hematocrit. Fewer corrected values are shown, because hematocrit was not determined on all occasions

dency for prothrombin values to be somewhat higher than thrombotest values

To investigate whether the slight discrepancy between thrombotest and prothrombin in the newborns could be explained by the presence of an inhibitor as has been shown for dicumarol treated adults (18) plasma samples from 3 newborns and mixed normal adult plasma were analyzed with thrombotest according to Hemker (18) The results are shown in Fig 3 As can be seen there was no evidence for the presence of an inhibitor

The correlation between prothrombin and thrombotest values is also illustrated in Figs 4 5 6 and 7 in which data from individual infants during the course of treatment with vitamin K and plasma are shown Effect of treatment with plasma and vitamin K was equally well registered with both methods

Prothrombin and thrombotest in normal full term newborns

Prothrombin and thrombotest values from 61 healthy full term newborns aged 0–24 hours are presented in Fig 8 As can be seen the distribution of prothrombin and thrombotest values was slightly skew The logarithms of the corrected values were normally distributed according to the normal equivalent deviate method (NED) (26) The mean value and mean ±2 S D calculated from the logarithmic values was for prothrombin 47% and 26–85 for thrombotest 45° and 27–74 . No values were found below 20° in our normal material

Prothrombin and prothrombin proconvertin during the first year of life

Prothrombin and prothrombin proconvertin (P P) values from 98 healthy children 0–12

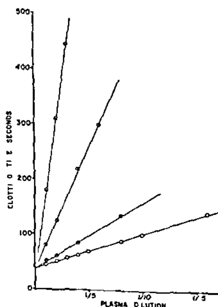


Fig 3 Plasma in different dilutions from neonates and adults was added to thrombotest reagent and the clotting time determined Clotting time was plotted against dilution ◓ ◒ ◑ individual neonates ○ mixed plasma from 20 normal adults

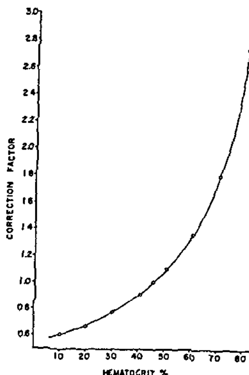


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The investigation was with short interruptions continued over a period of one year Samples were taken only on week days during day time

RESULTS

Correlation between prothrombin and thrombotest values

In Fig 2 is shown the correlation between prothrombin and thrombotest values for the entire clinical material with and without correction for hematocrit As can be seen there is a ten

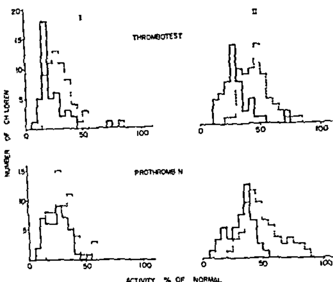


Fig 8 Prothrombin and thrombotest values from 45 hospitalized neonates, aged 0–24 hours, and from 61 healthy full term neonates aged 0–24 hours (with dotted lines) I values not corrected for hematocrit II values corrected for hematocrit

ence was significant ($p<0.01$) only for prothrombin values As the hematocrit for the hospitalized neonates was significantly lower ($p<0.001$) than the hematocrit for the healthy infants correction for hematocrit increased the difference between the groups

In the hospitalized neonates there was no significant correlation between birth weight and prothrombin or thrombotest values nor any sex difference Correlation between gestational age and prothrombin values was significant ($p<0.01$) but there was no correlation between gestational age and thrombotest values

15 of the hospitalized neonates aged 0–24 hours (Table 2) and 5 of the infants aged 1–21 days had a corrected prothrombin or thrombotest value of 20% or lower This low number could probably be explained by the intense treatment Some infants were treated with vitamin K and/or plasma even before samples could be taken for the combined investigation of prothrombin and thrombotest

In our clinical material there were only two infants with manifest bleeding symptoms One was a boy aged 1 day with respiratory distress syndrome He had a prothrombin value of 18 and a thrombotest value of 22% The

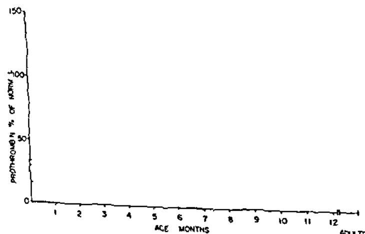


Fig 9 Prothrombin values not corrected for hematocrit, for healthy children during the first year of life.

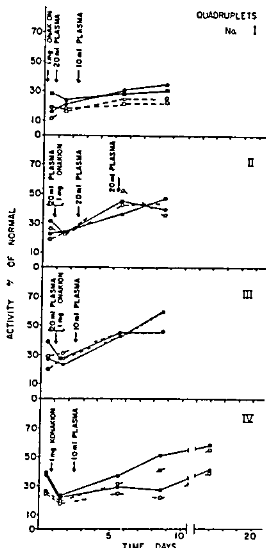


Fig 4 Quadruplets not hormon induced Gestational age 30 weeks I Immature child Birth weight 1 260 g II Slightly asphyctic child Birth weight 1 190 g III Slightly asphyctic child Birth weight 990 g IV Child with moderate respiratory distress syndrom Birth weight 1 210 g
o prothrombin values not corrected for hematocrit • prothrombin values corrected for hematocrit □ thrombotest values not corrected for hematocrit ■ thrombotest values corrected for hematocrit

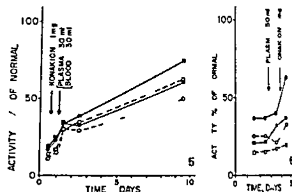


Fig 5 Child of diabetic mother Gestational age 37 weeks Delivery with cecaerean section Birth weight 1 950 g Moderate respiratory distress syndrom o prothrombin values not corrected for hematocrit, • prothrombin values corrected for hematocrit □, thrombotest values not corrected for hematocrit ■ thrombotest values corrected for hematocrit.

Fig 6 Small for date dysmature child Gestational age 41 weeks Birth weight 2 430 g o prothrombin values not corrected for hematocrit • prothrombin values corrected for hematocrit □ thrombotest values not corrected for hematocrit ■ thrombotest values corrected for hematocrit

months old, are shown in Figs 9 and 10 These values were not corrected for hematocrit As can be seen prothrombin values reach the adult normal range at the age of 6–12 months and prothrombin proconvertin activity at the age of about 3 months

Prothrombin and thrombotest in hospitalized newborns

Data from the hospitalized neonates aged 0–24 hours, 45 in all, are summarized in Fig 8 The values from the healthy full term neonates are shown for comparison Using values corrected for hematocrit the mean ±2 S D for the hospitalized neonates was for prothrombin 30% ±27% and for thrombotest 31% ±21% The hospitalized neonates had significantly lower corrected prothrombin and thrombotest values than the normal full term infants ($p<0\ 001$) For uncorrected values the differ

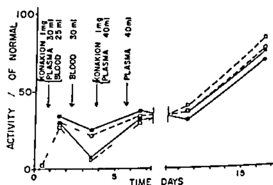


Fig 7 Child of mother with toxicosis Gestational age 33 weeks Severe respiratory distress syndrom o prothrombin values not corrected for hematocrit • prothrombin values corrected for hematocrit □ thrombotest values not corrected for hematocrit ■ thrombotest values corrected for hematocrit.

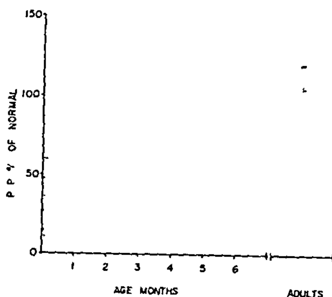


Fig 10 Prothrombin–proconvertin (P-P) values not corrected for hematocrit for children during the first year of life

other infant was a girl, aged 3 days with transposition of the great vessels She had a prothrombin value of 15% and a thrombotest value of 6% (all values corrected for hematocrit) Both infants were severely asphyctic

DISCUSSION

In the present investigation a close correlation was found between prothrombin concentration specifically determined, and thrombotest activity in the newborn child Thrombotest values however, tend to be slightly lower than prothrombin values In the dicumarol treated adult patients thrombotest gives lower values than specific tests for separate coagulation factors including prothrombin (18) It has been shown that the presence of an inhibitor (18) influences thrombotest in these cases In the newborn no such inhibitor could be demonstrated As thrombotest registers deficiencies of all the factors of the prothrombin complex (factors II, VII, IX and X) and is most sensitive to changes of factors VII and X (32), it seems probable that in many cases factor VII or X is more depressed than prothrombin itself, resulting in lower thrombotest than prothrombin values Other investigators, using one and two-stage assays of prothrombin, have demonstrated somewhat higher prothrombin than factor VII values in the newborn (24 39) According to Fresh et al (11) the relation between prothrombin and factor VII in the newborn child depends on the amount of vitamin K given and how early the treatment is started In our material too few infants were followed during treatment with vitamin K in varying doses to allow any conclusions about relations of prothrombin and factor VII values ex

Table 2 *Infants aged 0–24 hours with prothrombin or thrombotest values (corrected for hematocrit) of 20% or lower*

Sex	Patient	Prothrombin Uncorr value	Prothrombin Corr value	Thrombotest Uncorr value	Thrombotest Corr value	Diagnosis
♀	B	13	14	5	—	Asphyxia immaturity
♀	S I	10	16	16	25	Asphyxia immaturity twin
♀	S II	11	9	17	14	Asphyxia immaturity twin
♂	C	12	21	8	14	Asphyxia severe
♂	L	21	25	17	20	Asphyxia
♂	M	8	10	12	16	Asphyxia immaturity
♂	S	17	25	12	17	Asphyxia
♂	H	15	18	12	14	RDS
♀	K	12	17	13	19	RDS
♀	L	8	12	17	25	RDS
♂	B	16	18	20	22	RDS intrapulmonal and intracerebral hemorrhages
♀	S I	11	17	19	29	Immaturity quadruplet
♂	C	24	29	15	18	Neonatal pneumonia
♂	A	17	19	20	22	Hypoalbuminemia
♂	L	27	36	14	19	Diabetic mother

pressed by thrombotest at different levels of vitamin K dosage However our results do not contradict the findings of van Meer (25) that during high speed synthesis of prothrombin and related factors factor VII values are higher than prothrombin values and at low speed synthesis factor VII values are lower than prothrombin values

Our finding that prothrombin reaches normal adult level at the end of the first year of life is in accordance with several other investigators (5 6 30) Prothrombin proconvertin values reach the normal adult level at the age of about 3 months as does factor VII according to Dyggve (9)

In contrast to several other investigators (13 22 40) we found it reasonable to correct prothrombin and thrombotest values for hematocrit as prothrombin and related factors are distributed in plasma and the hematocrit shows great variability in this period of age The distribution of prothrombin values thus corrected showed good agreement with the values obtained by Nihlen & Ganrot with an immunological assay in plasma samples from neonates (12 27)

The lower limit, calculated from the mean logarithmic value −2 S D of our normal full term neonates was for prothrombin 26 and for thrombotest 27 (corrected values) In our normal material no values were found below 20%

Bleeding complications were rare in our material probably because of intense treatment with vitamin K and plasma No manifest bleeding was observed in any patient with prothrombin above 20 and thrombotest above 22 (corrected values) In the bleeding cases influence of other factors than the prothrombin concentration might be of decisive perhaps additional importance

Among the cases with decreased prothrombin and/or thrombotest values children suffering from hypoxia prevailed Three of these children were given vitamin K 2 hours after instead of immediately after birth but this could hardly explain the finding A depressing effect of hypoxia on prothrombin and other coagulation factors has been reported and discussed by many authors (1 2, 8 14 15 17 20 21 37 and others) In recent animal experiments it has been shown (15) that prolonged periods of hypoxia in the newborn dog gives depression of factors II V VII and X

The three test methods used in our investigation are all of value for clinical use As the reagents for the prothrombin proconvertin method have to be prepared by each laboratory and stored at −20 C our interest has been focused on the prothrombin and thrombotest assays for which freeze-dried ready made reagents are supplied The prothrombin assay is carried out in two steps and is consequently somewhat more cumbersome technically than thrombotest which is a one stage method and easy to perform On the other hand the prothrombin assay might be used for the whole range of prothrombin concentrations while thrombotest is less reliable in the range 50–100% (33)

SUMMARY

Prothrombin concentration determined specifically with a new two-stage method was compared with thrombotest activity in samples from infants aged 0–21 days There was a significant correlation between the two methods

Effect of treatment with vitamin K and plasma was registered with both methods

As the distribution of prothrombin and thrombotest values from normal full term neonates was slightly skew mean value and S D was calculated from the logarithmic values The normal range expressed as mean ±2 S D was for prothrombin 26–85 and for thrombotest 27–74 (values corrected for hematocrit)

No bleeding was observed in any patient with prothrombin above 20% and thrombotest above 22 (corrected values) Most low values in newborns were found in children suffering from hypoxia

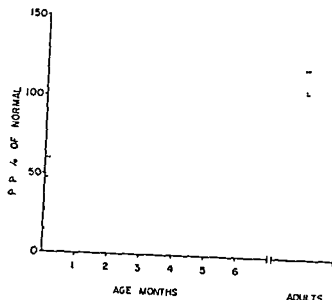


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Prothrombin concentration was also compared with prothrombin proconvertin activity in samples from children, aged 0–12 months In the healthy child prothrombin values reached the normal adult level at the age of 6–12 months, prothrombin proconvertin values at the age of about 3 months

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CIRCULATORY ADAPTATION IN THE THERMOREGULATION OF FULLTERM AND PREMATURE NEWBORN INFANTS

KRISTINA BERG and OLOV CELANDER[1]

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Important contributions to our knowledge of thermoregulation in the newborn have been made during recent years, both as regards the control of thermogenesis (2 8, 9) and the control of heat loss by regulation of cutaneous circulation (2) The meticulous work by Bruck and co workers is outstanding in this respect, and the reader is referred to the review by Bruck (2) for a full discussion of this subject In these latter studies skin blood flow was estimated by measurements of the thermal conductivity coefficient according to Hensel & Bender (7)

Since more quantitative methods to determine the rate of circulation in the extremities of newborns have become available important differences to the adult have been established (1 3, 4, 5 6) Such data provide a useful background when circulatory adjustments in thermoregulation are considered Besides giving information on the rate of blood flow in quantitative terms, the plethysmographic method may have certain advantages as the local effect on circulation may be studied separate from reflexogenic effects of induced temperature changes on remote parts of the body surface

The present report is based on observations obtained from a large number of infants carried out with the aim of examining at first hand the capacity of the newborn to control peripheral circulation in relation to the surrounding temperature

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MATERIAL AND METHODS

The majority of our subjects were fullterm infants which upon repeated examination by a paediatrician were considered normal and healthy They were taken from the wards to the laboratory preferably between their regular feeding hours. The laboratory was thermostatically maintained at 23 C A suitable plethysmograph was fitted to the right foot and calf after which the infant was put into an Isolette incubator The blood flow recording was performed as previously described (3) After a period of approximately 30 min the infants had mostly fallen asleep and the rate of limb blood flow had reached a steady level Series of blood flow determinations were then performed at short intervals as the temperature in the incubator (ambient temperature) or the temperature of the water within the plethysmograph (local temperature) or both these variables were gradually lowered

Temperature of the incubator

The temperature at the start of the experiment was kept at different levels within the range from 30 C to 35 C This was done in order to determine basal blood flow at different temperature levels (see Table 1) A gradual lowering of the temperature was achieved by turning off the heating filling the ice box with ice and letting the side holes of the incubator hood remain open A thermocouple was suspended just over the chest of the infant and the temperature within the incubator was continuously recorded on an electrothermometer We preferred to let the exposure of the child to a colder environment take some time rather then to create a more abrupt change since the final adaptation to a lower temperature was considered as the most important object of our study Therefore the fall of temperature was allowed to proceed at an approximate rate of 0 5 C per min

Temperature of the plethysmograph

The original method to maintain the temperature of the water in the plethysmograph constant (3) was altered to a system of water circulating through the

Table 1 *Resting limb blood flow at different levels of ambient temperature and local temperature (mean values from 131 fullterm healthy newborns)*

	Subgroup	No of infants	Ambient temp (C)	Local temp (C)	Limb flood blow (ml/min/100 ml)	SD
More than 12 hrs of age	A	57	30	34	7 3	±2.7
	B	40	33–35	34	8 2	±2 2
	C	15	30	40	8 9	±2 5
Less than 12 hrs of age	D	26	30	34	5 4	±2.5
	E	13	30	40	7 3	±2.6

plethysmograph from a reservoir outside the incubator The inlet of water from the reservoir and the outlet of water from the plethysmograph was adjusted by screw clamps, so that the volume of water in the plethysmograph remained constant. During the experiment the temperature of the water in the plethysmograph could thus be kept constant or it could be altered by this simple device This circulation of water through the plethysmograph was interrupted during the actual measurements of blood flow A thermocouple inserted into the plethysmograph provided a continuous recording of the temperature of the water in the plethysmograph. In the majority of children rectal temperature was also continuously recorded by way of a rectal thermocouple

General experimental conditions

All measurements were carried out on infants during natural sleep The exposure to a cold environment had a marked arousal effect. Each experiment was carried on until the infant became restless or woke up Changes in wakefulness and motor activities that sooner or later became prominent, were recorded

The following series of experiments are included in this report.

1 The resting blood flow was determined in 131 fullterm newborn infants at different combinations of ambient and local temperatures within the assumed "neutral zone This series is divided into five different subgroups, based on differences in temperature combinations and in postnatal age (see Table 1)

The circulatory response to a cold stimulus induced by a lowering of ambient temp rature local temperature or both was studied in additional 46 infants. These studies were divided into four main subgroups (see Table)

In 13 fullterm infants, ambient temperature was lowered whereas local temperature was maintained constant. Series A

3 In 8 fullterm infants, both ambient and local temperature was lowered Series B

4 In 17 fullterm infants, local temperature was lowered whereas ambient temp rature was maintained constant. In Series C ambient temp-rature was close to the lower limit, and in Series C close to the upper limit of the assumed neutral zone

5 Fourteen exposures to a lowering of ambient temperature were performed on 8 premature or low birthweight infants of different gestational and post natal age Series D and D_1.

There were no complications whatsoever while performing these studies

RESULTS

1 *Resting blood flow in the foot and calf at different levels of ambient temperature and local temperature*

Data obtained on measurements on 131 healthy fullterm newborns are summarized in Table 1 When flow was measured at 30 C ambient temperature and 34°C local temperature (group A) the mean value for this group was 7 3 ml/min/100 ml This is of the same order of magnitude that was found in previous series (3 5) Either an increase of ambient temperature (group B) or of local temperature (group C) raised the rate of local blood flow some 15 to 20 per cent The one conclusion that may be drawn is that both ambient temperature and local temperature influence the rate of peripheral blood flow to some extent and that this fact has to be carefully considered in studies on peripheral circulation

Group D and E in Table 1 refer to measurements during the first 12 hours of life These observations show that under identical temperature conditions the rate of blood flow is lower at this early stage but also that an increase of local temperature may still provoke a vasodilatation This restriction of peripheral circulation during the first hours of life also limits the maximal blood flow capacity at reactive hyperaemia (6)

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Important contributions to our knowledge of thermoregulation in the newborn have been made during recent years both as regards the control of thermogenesis (2, 8, 9), and the control of heat loss by regulation of cutaneous circulation (2) The meticulous work by Bruck and co workers is outstanding in this respect, and the reader is referred to the review by Bruck (2) for a full discussion of this subject In these latter studies skin blood flow was estimated by measurements of the thermal conductivity coefficient according to Hensel & Bender (7)

Since more quantitative methods to determine the rate of circulation in the extremities of newborns have become available, important differences to the adult have been established (1, 3 4, 5, 6) Such data provide a useful background when circulatory adjustments in thermoregulation are considered Besides giving information on the rate of blood flow in quantitative terms, the plethysmographic method may have certain advantages as the local effect on circulation may be studied separate from reflexogenic effects of induced temperature changes on remote parts of the body surface

The present report is based on observations obtained from a large number of infants carried out with the aim of examining at first hand, the capacity of the newborn to control peripheral circulation in relation to the surrounding temperature

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MATERIAL AND METHODS

The majority of our subjects were fullterm infants which upon repeated examination by a paediatrician were considered normal and healthy They were taken from the wards to the laboratory preferably between their regular feeding hours The laboratory was thermostatically maintained at 23 C A suitable plethysmograph was fitted to the right foot and calf after which the infant was put into an Isolette incubator The blood flow recording was performed as previously described (3) After a period of approximately 30 min the infants had mostly fallen asleep and the rate of limb blood flow had reached a steady level Series of blood flow determinations were then performed at short intervals as the temperature in the incubator (ambient temperature) or the temperature of the water within the plethysmograph (local temperature) or both these variables were gradually lowered

Temperature of the incubator

The temperature at the start of the experiment was kept at different levels within the range from 30 C to 35 C This was done in order to determine basal blood flow at different temperature levels (see Table 1) A gradual lowering of the temperature was achieved by turning off the h ating filling the ice box with ice and letting the side holes of the incubator hood remain open A thermocouple was suspended just over the chest of the infant and the temperature within the incubator was continuously recorded on an electrothermometer We preferred to let the exposure of the child to a colder environment take some time rather then to create a more abrupt change since the final adaptation to a lower temperature was considered as the most important object of our study Therefore the fall of temperature was allowed to proceed at an approximate rate of 0 5 C p r min

Temperature of the plethysmograph

The original method to maintain the temperature of the water in the plethysmograph constant (3) was altered to a system of water circulating through the

Table 3 *The circulatory response to lowered ambient temperature on 8 premature healthy infants (local temperature not maintained constant)*

No	Birth weight (g)	Gestat period (weeks)	Post natal age (days)	Temp of incubat. (C)	Temp of pleth. (C)	High temp blood flow (ml/min/100 ml)	Temp of incubat (C)	Low temp blood flow (ml/min/100 ml)	Ratio $\frac{\text{Blood flow at low temp}}{\text{Blood flow at high temp}}$
1	1 6.0	30	6	32.5	34	7 5	29	2.4	0 35
			17	32 5	34	7 5	28	3 5	0 45
			36	32	34	16 7	28	5 8	0 35
2	1 450	30	42	32	34	13 7	30	10 7	0 80
3	1 930	32	4	32	34	7 0	29 5	4 3	0 60
			22	32	34	16 9	29	3 5	0.[illegible]0
4	1 500	33	3	32.5	34	5 0	29	3 5	0 70
5	1 810	35	7	32.5	34	11 7	29	2.2	0.20
			15	33	36 5	17 4	28	6 1	0 35
6	1 830	37	5	32.5	34	4 5	27 5	3 4	0 75
			21	32.5	34	18 7	27 5	8 8	0 45
7	1 8[illegible]0	36	9	32	34	11 6	28 5	5 4	0 45
			23	32	34	12.4	30	7 3	0 60
8	1 4 0	33	21	32	34	23 0	26 5	6 8	0 30

30 C which is at the lower limit of the assumed "neutral zone The thermal stimulus was a progressive lowering of the temperature in the plethysmograph from the initial level of 35 C to around 26°C In this series there was a rather pronounced vasoconstrictor response from 6 4 to 2 6 ml/min/100 ml of tissue which is a highly significant difference As the stimulus of a lowered temperature was limited to the area from which flow determinations were made there are two possible mechanisms by which a vasoconstrictor response may be obtained In the first hand, a direct effect on the cutaneous blood vessels has to be considered And secondly there is the possibility that the reduction of blood flow is even under these experimental conditions at least partly due to a true thermoreflex over hypothalamic thermoregulating nervous structures That the latter mechanism is quantitatively the more important is shown by the findings in Series C_2 Here the local cold stimulus was much stronger as the temperature of the water in the plethysmograph was gradually lowered from 35 C to 15-22 C But despite this stronger local cold stimulus the reductions in blood flow in these infants were less prominent (from 6 6 to 5 0 ml/min/100 ml of tissue) The difference in experimental conditions in Series C_1 and C that explains this increased resistance to a local cold stimulus is the higher general ambient temperature that was applied to the infants in Series C An ambient temperature of 35°C (instead of 30 C) over the major part of the body surface obviously protects the infant from a more prominent reflex vasoconstriction even at a marked drop of local temperature (13–20 C) On the other hand, if ambient temperature is kept close to the lower limit of the "neutral" zone (30 C) a relatively much smaller change of local temperature on the foot and calf (8–10 C) may suffice to start a more prominent reflex vasoconstriction

5 *Circulatory response in premature infants to lowered ambient temperature local temperature not maintained constant*

This series was included to find out whether or not active thermoregulation is developed at premature birth In a more detailed study on premature infants (1) we have noticed that premature infants generally have a very high rate of limb circulation under resting conditions and also that the maximal blood flow capacity at reactive hyperaemia is correspondingly higher—both studies indicating a high vascularity We have also found that when prema-

Table 2 *The circulatory response to lowering of ambient temperature, local temperature, or both in four different experimental series Temperatures and mean values of local blood flow before and after exposures and the probability of the blood flow differences are given × marks when local temperature was allowed to cool with the ambient temperature*

Series	Subjects	n	Age	Temperature °C before ambient		Temperature °C after ambient		Blood flow (mean±S E ml/min/100 ml tissue) before	after	Probability of the flow difference
				ambient	local	ambient	local	before	after	
A	Normal healthy fullterms	13	6 hrs–5 days	32 5–35	35	25–28	35	8 2±0 8	5 2±0 4	$p<0\ 01$
B	Normal healthy fullterms	8	1–5 days	30–35	35	22–27	×	8 5±1 0	4 5±0 8	$p<0\ 01$
C_1	*Normal healthy* fullterms	7	*4 hrs*– 6 days	*30*	*35*	*30*	*25–27*	6 4±0 9	2 6±0 4	$p<0\ 01$
C_2	Normal healthy fullterms	10	4 hrs– 6 days	35	35	35	15–22	6 6±0 9	5 0±0 8	$p<0\ 05$
D_1	Healthy prematures	5	<8 days	32 5	34	27 5–29 5	×	7 1±1 3	3 1±0 4	$p<0\ 05$
D_2	Healthy prematures	9	>8 days	32 5	34	27 5–30	×	17 0±1 0	7 0±1 0	$p<0\ 01$

2 *Circulatory response to lowering of ambient temperature local temperature maintained constant*

Table 2, Series A gives the blood flow rates in 13 fullterm infants at an ambient temperature within neutral" zone and after a drop in ambient temperature of 4 5°–8°C The children differed in many respects, such as age resting blood flow prior to exposure to lower ambient temperature and the magnitude of temperature change which was tolerated until they awoke The general trend however is quite clear A lowering of ambient temperature of this magnitude provoked a definite decrease of blood flow (from 8 2 to 5 2 ml/min/100 ml of tissue which is a highly significant difference) The capacity to adjust the rate of blood flow through the limb according to the temperature of the surrounding air was evident already on the first day of life This type of experiment also proves that the reduction of blood flow was a true thermogenic reflex, since any local effect on the limb circulation was ruled out by maintaining the temperature of the water in the plethysmograph at 35°C throughout the period of exposure

3 *Circulatory response to lowering of ambient temperature local temperature not maintained constant*

In order to find out to what extent the effect of a lowered temperature was due to a local effect on the skin vessels a series of low-temperature exposures was carried out in which no efforts were made to keep local temperature in the plethysmograph at a steady level of 35°C as in Series A The results are given in Table 2 Series B The vasoconstriction is of the same order of magnitude as in Series A Obviously a lowering of the local temperature adds little to the reflex response of a lowering of ambient temperature Nor is it possible to counteract the general vasoconstrictor response by maintaining a high local temperature

4 *Circulatory response to lowering of local temperature ambient temperature maintained constant*

Two types of experiment were included in this series and the data obtained are summarized in Table 2 Series C_1 and C Seven infants (Series C_1) were kept at an ambient temperature of

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No	Birth weight (g)	Gestat. period (weeks)	Post natal age (days)	Temp of incubat. (C)	Temp of pleth (C)	High temp blood flow (ml/min/ 100 ml)	Temp of incubat (C)	Low temp blood flow (ml/min/ 100 ml)	Ratio Blood flow at low temp / Blood flow at high temp
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			22	32	34	16 9	29	3 5	0 [illegible]0
4	1 500	33	3	32.5	34	5 0	29	3 5	0 70
5	1 810	35	7	32.5	34	11 7	29	2.2	0 20
			15	33	36 5	17 4	28	6 1	0 35
6	1 830	37	5	32.5	34	4 5	27 5	3 4	0 75
			21	32.5	34	18 7	27 5	8 8	0 45
7	1 8[illegible]0	36	9	32	34	11 6	28 5	5 4	0 45
			23	32	34	12 4	30	7 3	0 60
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ture infants have been examined repeatedly at various postnatal ages, the blood flow values are considerably higher in a later period, when the infant is gaining weight, than early, when body weight is still falling Irrespective of the background to this intense peripheral blood flow and its subsequent further increase among prematures, it is obvious that these higher rates of blood flow, together with the higher surface area/bodyweight ratios in prematures are heat-loss promoting factors, which must be taken into consideration in studies on thermoregulation in prematures

Table 3 summarizes fourteen exposures to lowered ambient temperature performed on 8 premature infants Most of these infants were subjected to repeated examination In Table 2, Series D_1 and D , the flow determinations have been grouped according to postnatal age It is quite obvious that the general level of blood flow is markedly related ($p < 0\ 01$) to postnatal age

Only a relatively small temperature change could be induced in these premature infants without producing an arousal effect As seen from Table 3 most exposures had to be interrupted at an ambient temperature level of 28–29°C At this temperature the infant became increasingly restless and finally woke up making any further measurements of peripheral circulation less reliable However before these arousal effects became prominent, limb circulation was invariably reduced by active vasoconstriction As seen from the data in Table 3 a lowering of ambient temperature of only 3–4°C produced an average decrease of limb circulation to about 50 per cent of initial level Thus even at advanced prematurity and irrespective of postnatal age there were no signs of impaired thermoregulatory control of cutaneous circulation

Fig 1 illustrates the high resting blood flow in a premature infant at a postnatal age of 3 weeks A gradual drop in ambient temperature from 32°C to 26 5°C over 2 hours brought about an extensive flow reduction to less than one third

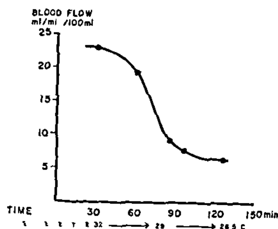


Fig 1 The circulatory response to a cold stimulus in a 3 week old prematurely born infant (gestational age 33 weeks 1 420 g) Note the extensive and smooth reduction in blood flow of the foot and calf See also Table 3

DISCUSSION

The present report is based on a large number of blood flow measurements at different temperature conditions The data obtained show that the rate of circulation in the foot and calf is highly dependent on the temperature to which the child may be exposed Any deviation from the neutral zone immediately brings about a reflex change of peripheral circulation by which heat loss from the body surface is adjusted to the altered temperature of the child s surroundings This circulatory adaptation has been shown to be a true thermoregulatory reflex, since in the present study the local temperature at the area from which the blood flow records were obtained could be maintained constant The local effect of cooling must be relatively less important since it was largely abolished if ambient temperature was kept high It should also be stressed that the reductions in peripheral circulation that may be achieved are quantitatively important

The present study also confirms our previous observations (3 6) that in the first hours of life the sympathetic tone on peripheral blood vessels is higher than later in the neonatal period At this early age the responsiveness to thermal stimuli is also less prominent The probable explanation is that as important adaptive processes are still going on the vas-

cular bed may be preoccupied with other central or reflex nervous influences over the vasomotor nerves The vast vascular supply that characterizes the premature infant will be discussed elsewhere (1) In this connection we would only like to stress the high basal blood flow which is typical for the premature infant in the later phase of increasing body weight Not only has such a premature infant an unfavourable surface to bodymass ratio the blood flow per unit surface must also be considerably bigger

The effect of a colder environment on circulation is by no means limited to a decrease of volume flow through the extremities The plethysmographic technique is basically a method to register volume and volume changes We have noticed repeatedly that exposure to a colder environment is followed by a measurable decrease of limb volume indicating that the capacity function of the vascular bed is also interfered with

Our studies were confined to sleeping infants and were interrupted as the infants woke up However it was quite clear that arousal is part of the effect of a colder environment on the infant as a whole The critical temperature at which arousal became prominent was in the majority of cases around 27–29 C At higher temperatures 31–34 C most infants were at thermal comfort Few children tolerated a temperature of 22–23 C without being awakened It would perhaps be possible to induce a greater temperature change than this provided this change was more rapid The behaviour at the arousal from sleep at thermal comfort was quite typical The first sign was an increase of flexor tone combined with short flexor movements During progressive exposure to a colder environment this increase of muscular activity was accompanied by gross muscular movements and bouts of crying for 10 to 30 sec The infant then became calm again for 1 to 2 min after which new series of increasing muscular activity appeared with increasing frequency Definite shivering was not observed but the described pattern of movements should probably be looked upon as an equivalent to shivering, and therefore as an integral part of thermoregulation at early age

The present observations on the rate of peripheral blood flow and the previous findings by Bruck and co-workers (2) on skin temperature show that the lability of body temperature well known for newborns and especially pronounced for the prematurely born cannot be attributed to a non functioning thermoregulatory control of peripheral circulation Other factors such as a large body surface being richly vascularized poor insulation by subcutaneous fat, short distances from the surface to core and a relatively larger insensible perspiration may account for greater heat losses when the newborn is contrasted against the adult Likewise recent evidence (2 8) shows that thermogenesis too is a prominent part of the thermoregulating mechanism in the newborn Active thermogenesis in the newborn is probably largely restricted to skeletal muscle The simple fact that skeletal muscle comprises a far smaller proportion of body mass in the newborn (and especially in the premature) than in the adult may pose another handicap for the newborn

SUMMARY

Blood flow measurements by venous occlusion plethysmography were performed on 131 sleeping healthy fullterm newborns at various external temperature conditions The peripheral circulation varies among different individuals but a definite relationship between ambient temperature and the rate of peripheral circulation could nevertheless be established

Different series of observations on exposure to acute changes of temperature conditions on a further 46 newborn infants allow the following conclusions

The control of peripheral circulation in relation to the temperature of the surroundings is reflex in nature and independent of the local effects of a cold stimulus

Circulatory changes are quantitatively pro-

ture infants have been examined repeatedly at various postnatal ages, the blood flow values are considerably higher in a later period, when the infant is gaining weight, than early, when body weight is still falling Irrespective of the background to this intense peripheral blood flow and its subsequent further increase among prematures, it is obvious that these higher rates of blood flow, together with the higher surface area/bodyweight ratios in prematures are heat-loss promoting factors, which must be taken into consideration in studies on thermoregulation in prematures

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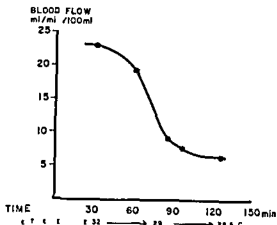


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NIEMANN PICK DISEASE AND GIANT CELL TRANSFORMATION OF THE LIVER

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Doubts have grown as to how many genotypically different syndromes are in reality encompassed by Niemann Pick disease The biochemical disturbance in over 160 recorded cases has been broadly classified as a *sphingomyelin sterol lipidosis* b cause of the accumulation of sphingomyelin together with cholesterol and glycero-phospholipids predominantly in tissue foam cells within the reticuloendothelial system There is however a broad range in the severity and distribution of such accumulations apart from great variations in the age of onset and clinical evolution with special reference to the timing and degree of CNS involvement But to what extent this heterogeneity of clinical and biochemical expression represents true genotypic variation remains to be resolved.

The first of the four clinico-pathological subdivisions of Crocker (3) is a rapidly progressive infantile form believed to typify 85 % of all th victims A classical pattern comprises an onset in early infancy ending in d ath by the age of 1 to 3 years a high incidence amongst Jews massive hepatosplenomegaly intense CNS involvement, and an association with an esp cially high sphingomyelin content of involved tissues Within this category examples of particularly early manifestations have presented themselves Infiltration was already grossly identifiable in the fœtus in two examples (2 12) and has been recognizable at birth or during the first month in more than 12 cases

Even amidst those already involved pre and neonatally the three affected siblings we document here may be representative of yet another specific grouping. Neonatal jaundice emerging by the 10th day was their prominent differential feature It can also be identified in the proformata of the two sibs of Maurer (15) three sibs of Jeune et al (10) three sibs of Crocker & Farber (3) and two of Ivemark et al (8) In one of the three examples cited by Fredericksson (4) jaundice persisted from shortly after birth whereas the other two did not quite conform in that neonatal jaundice lingered only 4 weeks in one and disappeared after a few days to reemerge after 7 weeks in the other It may be noted moreover that in addition to the background of consanguinous marriage in our cases and those of Crocker & Farber (3) and Ivemark et al (8) all cases presented with neonatal jaundice were in familial groups although the overall familial incidence for all cases of Niemann Pick was calculated by Fredrickson as not exceeding 25 % Likewise although he assessed the Jewish incidence of this disease at about 40 all the familial groups hitherto reported in our category had

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minent and do not support the view that thermolability in the newborn is due to a deficient thermocontrol of the cutaneous circulation

The circulatory thermoreflex operates even during the first day of life and is as prominent in premature infants as in fullterm infants

The importance of these observations in relation to other mechanisms deciding heat loss and thermogenesis in the newborn is briefly discussed

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the jaundice deepened, the child remained active with normal reflexes posture and psychomotor development. The weight gain was slow the abdomen was distended mainly because of the liver which extended 5 5 cm below the right costal margin (Fig 1)

Laboratory findings The serum bilirubin both direct and indirect, was estimated throughout the course of the illness (Fig 2) It can be seen that the bilirubin level was 18 mg/100 ml admission rising to 23 mg/100 ml within a few days On prednisone therapy the serum bilirubin decreased to 15 mg/100 ml When the prednisone was reduced to 40 mg/day the level rose again to 21 mg/100 ml a fall to 15 mg/100 ml following upon a return of the prednisone dosage to 60 mg day On tapering off the steroid administration the bilirubin rose gradually and before operation was 32 8 mg/100 ml

The SGOT on admission was 162 and gradually rose to 240 units the SGPT on admission was 10 rising gradually to 0 and then more sharply to 146 units Total protein ranged between 5 4-6 8 mg/100 ml Serum cholesterol was between 195 and 284 mg/100 ml Thymol turbidity on admission was 1 8 units the thymol flocculation was negative and cephalin flocculation was + + later on the thymol turbidity rose to 9.2 units, thymol flocculation to + + + + whereas the cephalin flocculation remained + +

Hypochromic anaemia developed during the course of the illness with the haemoglobin declining from 11 4 to 7 4 g/100 ml Leucopenia was not prominent nor was there thrombocytopenia Leucocyte counts were between 7 100 and 4 950/mm thrombocytes 760 000 mm No Niemann Pick cells were detected in the peripheral blood smears nor in the bone marrow

An exploratory laparotomy was performed at which a biopsy was taken from the liver This was examined histologically electron microscopically and biochemically

Frozen and parrafin embedded sections were examined The liver showed a distortion of the lobular pattern and an occurrence of many parenchymatous giant cells There were bile casts in the canaliculi and pigment within the giant cells Some liver cells were swollen and so were some sinusoidal cells There was an increase in the fibrous tissue in portal areas and in between the liver cells. Bile ductules and ducts in portal areas appeared scanty in number

A lipidosis was not suspected, and only on re examining the slides after the baby s death, was it realised that the sinusoidal cells were indeed lipid laden Niemann Pick cells.

Electron microscopic examination showed the liver cells to contain many myelin figures and lobulated acuole like structures (Fig. 10) (19)

Enzyme studies of the biopsy material showed marked decrease of glucuronyl transferase activity UDPG glucuronyl transferase (EC 2 4 1 17) was assayed with 4 methylumbelliferone as substrate by a modification of the method of Arias (1) Glucose 6-Phosphatase was assayed according to Hers (6) Glycogen was determined by an enzymatic method employing amyloglucosidase and glucose oxidase (9) after alkaline hydrolysis and precipitation with ethanol

RESULTS

	Glucuronyl transf (nmol/mg prot/min)	Normal
Case 1		
Liver biopsy	1 12	8 7±0 6
Liver PM	0 64	
Kidney PM	5 02	
Case 3		
Liver biopsy	0 99	

Liver glycogen concentration in the biopsy obtained from case 1 was 1 2 % In the case of case 3 another microsomal enzyme glucose-6 phosphatase was also determined Activity of this enzyme was 57 nmol/mg prot/min (controls 39±2)

The low activity of liver glucuronyl transferase was in contrast to the normal activity found in the kidney of case 1 and could not be related to the apparently unimpaired ability of the liver to conjugate bilirubin as evidenced by the elevated serum level of direct reacting bilirubin

In vitro inhibition of UDPG glucuronyl transferase by bilirubin has recently been described but it seems improbable that such an inhibition was the cause of the low enzyme activity observed (5) the concentrations at which bilirubin inhibited the reaction were extremely high whereas in our assay the final concentration of bilirubin in the reaction mixture did not exceed 0 5 mg/100 ml Results obtained with different amounts of homogenate in the assay giving final bilirubin concentrations ranging from 0 5 mg/100 ml were identical

The question whether there exists only one enzyme glucuronidating the various substrates used for assay of UDPG glucuronyl transferase in addition to bilirubin has not been finally settled

Although quantitative differences in the activity of human liver enzyme towards various



Fig 1 Case 1 At the age of 2 months showing enormous enlargement of liver and spleen

been described as non Jewish Of other components shared, at least in our case and those of Crocker and Ivemark, an interesting aspect is the hepatic giant cell transformation which in several instances led indeed to initial confusion with giant cell hepatitis and cirrhosis because of the coexisting fibrosis and cholestasis

The relationship of jaundice would be worthy of further study Despite previous claims of full functional normality serial measure of the transaminase especially of SGOT, revealed early evidence of parenchymal cell damage or dysfunction Our parallel finding of almost absent glucuronyl transferase activity in the liver biopsy material could account, however for only a small proportion of the early and persistent jaundice, since the total serum bilirubin in our infants embodied a relatively high proportion of the direct or conjugated form (Fig 2)

DESCRIPTION OF CASES

Family history The parents are Persian Jews related to each other the father being the mother's uncle Both are healthy with no past history of serious illness or jaundice Their first child was born in 1964 a normal healthy girl who is now 6 years old The second child also a girl (our case 2) was born in 1965 and died at the age of 3 months at another hospital The third child a girl was born at term in August 1966 and is our case 1

In 1969 a full term boy (case 3) was born and developed a clinical picture similar to the two previous siblings liver biopsy again showing sphingomyelin lipidosis and giant cell transformation This child also died at age of 3 months and no permission for autopsy could be obtained

Case 1

Clinical picture A girl weighing 2 700 g was born at term after a normal pregnancy The immediate neonatal period was uneventful except for the appearance of jaundice on the second day of life which increased for 2 days and disappeared by the sixth day of life only to reappear 8 days later in association with yellowish brown urine and pale stools Although

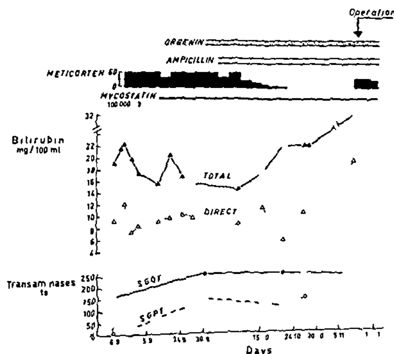


Fig 2 Case 1 *Bilirubin and transaminase estimations*

the jaundice deepened the child remained active with normal reflexes posture and psychomotor development The weight gain was slow the abdomen was distended mainly because of the liver which extended 5 5 cm below the right costal margin (Fig 1)

Laboratory findings The serum bilirubin both direct and indirect, was estimated throughout the course of the illness (Fig 2) It can be seen that the bilirubin level was 18 mg/100 ml admission rising to 23 mg/100 ml within a few days On prednisone therapy the serum bilirubin decreased to 15 mg/100 ml When the prednisone was reduced to 40 mg/day the level rose again to 21 m /100 ml a fall to 15 mg/100 ml following upon a return of the prednisone dosage to 60 mg day On tapering off the steroid administration the bilirubin rose gradually and before operation was 32 8 mg/100 ml

The SGOT on admission was 162 and gradually rose to 240 units the SGPT on admission was 10 rising gradually to 20 and then more sharply to 146 units Total protein ranged between 5 4-6 8 mg/100 ml. Serum cholesterol was between 195 and 284 mg/100 ml Thymol turbidity on admission was 1 8 units the thymol flocculation was negative and cephalin flocculation was + + later on the thymol turbidity rose to 9 2 units, thymol flocculation to + + + + whereas the cephalin flocculation remained + +

Hypochromic anaemia developed during the course of the illness with the haemoglobin declining from 11 4 to 7 4 g 100 ml Leucopenia was not prominent, nor was there thrombocytopenia Leucocyte counts were between 7 100 and 4 950/mm thrombocytes 460 000 mm No Niemann Pick cells were detected in the peripheral blood smears nor in the bone marrow

An exploratory laparotomy was performed at which a biopsy was taken from the liver This was examined histologically electron microscopically and biochemically

Frozen and parrafin embedded sections were examined The liver showed a distortion of the lobular pattern and an occurrence of many parenchymatous giant cells There were bile casts in the canaliculi and pigment within the giant cells Some liver cells were swollen and so were some sinusoidal cells There was an increase in the fibrous tissue in portal areas and in between the liver cells Bile ductules and ducts in portal areas appeared scanty in number

A lipidosis was not suspected and only on re examining the slides after the baby s death, was it realised that the sinusoidal cells were indeed lipid laden Niemann Pick cells.

Electron microscopic examination showed the liver cells to contain many myelin figures and lobulated vacuole like structures (Fig 10) (19)

Enzyme studies of the biopsy material showed marked decrease of glucuronyl transferase activity UDPG glucuronyl transferase (EC 2 4 1 17) was assayed with 4 methylumbelliferone as substrate by a modification of the method of Arias (1) Glucose 6-Phosphatase was assayed according to Hers (6) Glycogen was determined by an enzymatic method employing amyloglucosidase and glucose oxidase (9) after alkaline hydrolysis and precipitation with ethanol

RESULTS

	Glucuronyl transf (nmol/mg prot/min)	Normal
Case 1		
Liver biopsy	1 12	8 7±0 6
Liver PM	0 64	
Kidney PM	5 02	
Case 3		
Liver biopsy	0 99	

Liver glycogen concentration in the biopsy obtained from case 1 was 1 2% In the case of case 3 another microsomal enzyme glucose 6 phosphatase was also determined Activity of this enzyme was 57 nmol/mg prot/min (controls 39±2)

The low activity of liver glucuronyl transferase was in contrast to the normal activity found in the kidney of case 1 and could not be related to the apparently unimpaired ability of the liver to conjugate bilirubin as evidenced by the elevated serum level of direct reacting bilirubin

In vitro inhibition of UDPG glucuronyl transferase by bilirubin has recently been described but it seems improbable that such an inhibition was the cause of the low enzyme activity observed (5) the concentrations at which bilirubin inhibited the reaction were extremely high whereas in our assay the final concentration of bilirubin in the reaction mixture did not exceed 0 5 mg/100 ml Results obtained with different amounts of homogenate in the assay giving final bilirubin concentrations ranging from 0 5 mg/100 ml were identical

The question whether there exists only one enzyme glucuronidating the various substrates used for assay of UDPG glucuronyl transferase in addition to bilirubin has not been finally settled

Although quantitative differences in the activity of human liver enzyme towards various



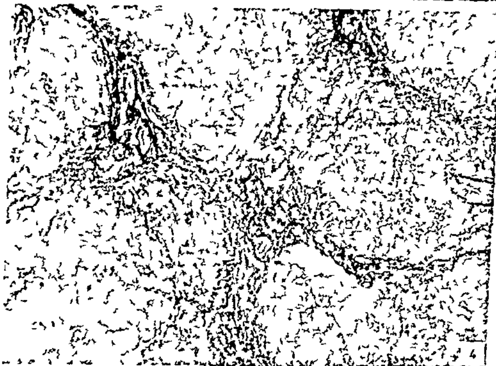


Fig 3 Case 1 Liver form cells giant cells and interstitial fibrosis H and E × 147

Fig 4 Case 1 Area of liver cirrhosis Azan × 43

substrates have been observed results with 4 methylumbelliferone have been in line with those obtained with O aminophenol or bilirubin as acceptor (1) The main difference between the assay with 4 methylumbelliferone and other substrates was in the greater sensitivity of the former, for this reason trace activities were obtained with umbelliferone in cases in which no activity could be detected with other substrates (1)

Activation of UDPG glycuronyl transferase activity towards various substrates by *in vitro* treatment of enzyme preparations with detergents has recently been described (13) It has been proposed that addition of detergents and other procedures causing *in vitro* activa



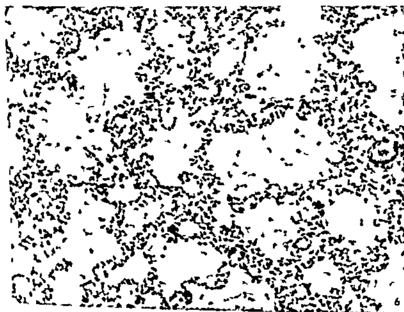


Fig 5 Case 1 Thymus almost unrecognisible because of lipid deposition within cells H and E × 43

Fig 6 Case 1 Lungs with foam cells in alveoli H and E, × 147

tion (7) lead to an unfolding of microsomal membranes which makes the substrate accessible to th enzyme (16) If this is the case the low enzyme activity found in the present cases may possibly be due to changes in the cell structure connected with the underlying disease which make the enzyme less accessible to the substrate Such a phenomenon in a crude non activated homogenate would not necessarily reflect the *in vivo* situation and explain the apparent discrepancy b tween the unimpaired ability to conjugate bilirubin *in vivo* and the low *in vitro* enzyme activity observed

Although there was no bleeding tendency the baby developed haemorrhagic ascites and died 5 days after the operation aged 3 months



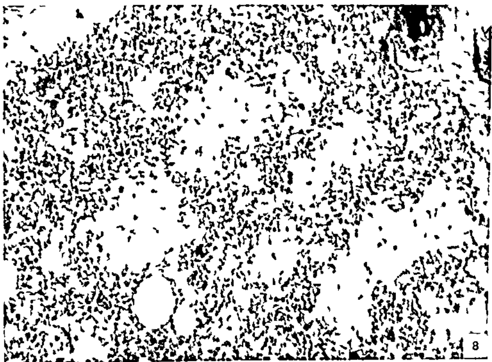


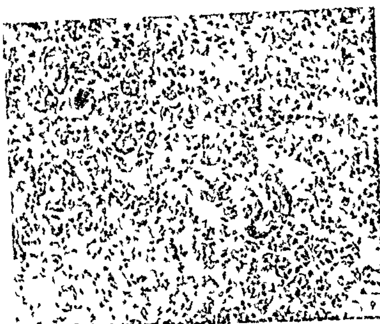
Fig 7 Case 2 Thymus with lipid deposition in cells H and E ×108

Fig 8 Case 2 Lungs with foam cells in alveoli H and E ×108

Autopsy

Gross findings The peritoneal cavity contained 80 ml of bloody fluid and some clotted blood around the site of the operative liver biopsy The liver was enlarged firm and greenish in colour, its surface and sections were unevenly granular The extra hepatic bile ducts were patent and normal the gall bladder was rather small and contained a small amount of yellowish viscous fluid The spleen weighed 120 g was fairly firm dark red in colour with vaguely defined paler areas The lymph glands and thymus were small The lungs were well aerated but had near their periphery small, dark red consolidated areas The other organs, including the brain showed no gross abnormalities

Microscopic findings Formalin and Zenker fixed tissue blocks were first examined When



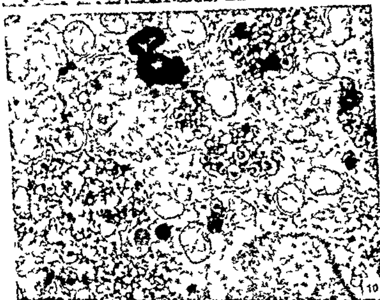


Fig 9 Case 2 Liver with giant cells and fibrosis H and E, × 147

Fig 10 Electron micrograph of the liver biopsy of case one The changes are non specific There are lobulated vacuolelike structures in the parenchymal cells (19)

it became obvious that the condition was a lipidosis, the stored organs were transferred to Baker s solution after 2 months in 10% formalin and frozen tissue sections were used for the various lipid stains.

The liver Numerous sections were examined and these presented varied appearances Some areas preserved a fairly normal liver architecture showing only an increase in connective tissue in portal areas and between liver cords and marked canalicular and intracellular bile stasis. These areas had normal bile ducts of various sizes in the portal tracts In other areas there was a marked giant cell transformation of liver cells with total loss of normal architecture and paucity of bile ducts The sinusoidal cells as well as some liver cells were often foamy too (Fig 3) so that it was difficult at times to distinguish between them In many regions definite cirrhosis with regenerative nodules could be seen (Fig. 4)

The reticuloendothelial system The spleen,



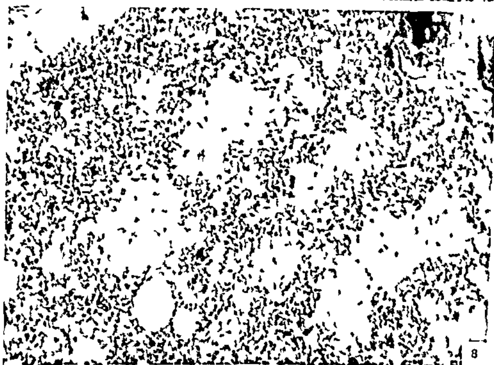


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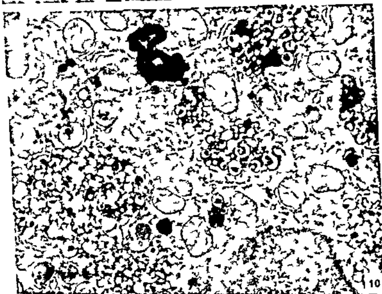


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The reticuloendothelial system The spleen

Table 1 *Results of histochemical procedures compared with the reactions usually obtained in Niemann Pick disease (14, 20)*

− = negative ± = slightly positive or variable + = positive + + = strongly positive ND = not done

	Case 1			Case 2			
	Lung	Liver	Spleen + thymus	Lung	Liver	Spleen + thymus	Niemann Pick disease
Sudan red (with heating)	±	±	−	−	+	−	±
Sudan black	+ +	+ +	+ +	+ +	+ +	+ +	+ +
Schultz method for cholestrol	+	ND	+	+	ND	±	+
Nile blue for fatty acids	+	ND	+	ND	ND	ND	+
Luxol blue (myelin stain)	±	+	−	ND	ND	ND	±
Smith-Dietrich method for phospholipids	+ +	+ +	+ +	ND	ND	ND	+
Bakers procedure for phospholipids (with pyridine extraction)	+ +	+ +	+ +	+ +	+ +	+ +	+
Toluidine blue metachromasia (PH7)	ND	+	+	ND	+	+	±
P A S + Diastase	ND	+	±	ND	±	±	±

thymus, lymph glands, bone marrow and Peyers patches contained large numbers of typical Niemann Pick cells The thymus was *almost totally replaced by foam cells (Fig 5)*

The lungs In several areas typical Niemann-Pick cells filled the alveoli (Fig 6) Lipid laden cells also occurred occasionally within the septa

Foam cells were also found in many kidney glomeruli at junction of cortex and medulla of the adrenals and in the lamina propria of the intestines

The central nervous system Many neurons in the spinal cord brain stem and basal ganglia were ballooned, with foamy cytoplasm and in distinct cellular borders These however, stained negatively for lipids

Case 2

A sister of case 1 died 1 year previously at another hospital also at the age of 3 months after a very similar clinical course the jaundice appeared at the age of 10 days deepened gradually alongside with progressive *hepatosphlenomegaly* The *post mortem diagnosis* were viral giant cell hepatitis interstitial pneumonia and malnutrition The formalin fixed organs of this case were available for renewed study

Microscopic examination of the spleen lymph nodes thymus and *lungs showed large numbers of* typical foam cells (Figs 7 and 8) The liver presented parenchymatous giant cell transformation fibrosis and foamy Kupfer cells (Fig 9)

In spite of the prolonged formalin *fixation* (18 months) it was possible to demonstrate pronounced lipid storage and a positive Baker s test for phospholipids in the liver thymus lymph glands spleen and lungs that this too was a case of Niemann Pick disease together with a giant cell transformation of the *liver*

The histochemical tests performed in both cases as well as the reactions usually obtained in Niemann Pick disease are summarised in Table 1

DISCUSSION

Especially striking clinico pathological analogies can be drawn between the three siblings *herewith described and the two siblings* of Ivemark (8) But such a resemblance albeit less complete, can readily be extended to the familial groups of Maurer (15) Kune (10), Crocker & Farber (3), and Frederickson (4) A very early postnatal onset almost certainly *following already prenatal involvement* was highlighted by their neonatal jaundice This *persisted and was marked by a sustained high level of conjugated or direct serum bilirubin* The infatile course was fulminant with early and progressive hepatosplenomegalv underlying typically conspicuous abdominal protuberance This was also attended by feeding difficulty with recurrent vomiting and an overall failure to thrive Death usually ensued within 3 to 9 months

Pathologically, another impressive analogy lay in the accompanying hepatic giant cell

transformation fibrosis and cholestasis invit ing a firm preliminary diagnosis of viral giant cell hepatitis in several examples The initial clinical expresssion of the familial form of Niemann Pick with its early and conspicuous hepatic component usually sufficed to over shadow the clinical picture and conceal the true nature of the disorder

Add to this laboratory evidence of paren chymal liver damage as well as cholestasis and the diagnostic diversion could be com plete *Significantly abnormal liver function tests are usually expected however only in ter minal phases With respect to thymol turbidity* and cephalin flocculation tests this was also true at least of our case 1 But of the serum transaminases whilst SGPT levels were not raised the SGOT was clearly incre_sed from an early stage Strangely enough this particu lar disparity between the serum transaminase findings has previously been noted in several examples of another storage disorder Tay Sachs disease (17)

Electron microscopy of liver biopsy material may clarify the underlying diagnosis but spe cifically oriented lipid histoch mistry can also readily identify the sphingomylin sterol laden foam cells in various organs With regard to either the Niemann Pick disorder or hepatic giant cell transformation there has been evi dence that severe involvement can already arise prenatally even perhaps dating back to the first trimester But rather than suggesting a primary and even associated gen~tically-de termined disturbance it is increasingly suspec ted that hepatic giant cell transformation ex presses a non specific reaction characterizing the immature parenchyma of the developing liver (4 8 11 18) In our sibling as well as in those of Ivemark et al (8) it presum ably reflected a secondary response to the con comitant Niemann Pick defect

Lung involvement is also uniformly typical of this disease Foam cells may fill the alveoli although there is nonetheless disproportionately slight impairment of respiratory function The radiological stigmata of a diffuse reticular or of a widespread nodular pattern may suggest a chronic interstitial pneumonia, as was both the pre and post mortem interpretation in the second case A helpful diagnostic measure could be the analysis of gastric aspirates for pathognomonic foam cells

SUMMARY

This report presents three siblings with neo-natal jaundice who died before the age of three months They were shown on autopsy to be suffering from Niemann Pick diseas_ together with a giant c ll transformation of the liver

The resemblance of these cases to previously reported examples of the above mentioned double condition is stressed and a suggestion is made that this variant of the Niemann-Pick disease presents a special genotypic syn-drome within the group with the biochemical disorder broadly classified as sphingomyelin lipidosis

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CARDIOMEGALY IN ASSOCIATION WITH NEONATAL HYPOGLYCAEMIA

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Hypoglycaemia is a well recognised complication in infants of low birth weight in relation to gestational age and in infants of diabetic mothers in the immediate neonatal period Various symptoms have been described occurring in association with hypoglycaemia apnoea convulsions lethargy and difficulty with feeding (4)

In the Hospital for Sick Children Toronto Dr George Collins (now in Montreal) first brought to our attention the occasional association of heart failure with neonatal hypoglycaemia Dr Bernard Reilly independently noted the same association with increased radiological heart size and pulmonary oedema

The purpose of this paper is to report the incidence and clinical course of this condition over a 12 month period between February 1968 and February 1969 We have found 6 infants who had hypoglycaemia tachypnoea (respiratory rate >65/min) and radiographic cardiac enlargement and signs suggestive of pulmonary oedema unassociated with other organic disease

MATERIAL AND METHODS

The Neonatal Division of the Hospital for Sick Children, Toronto dealt with more than 1 000 patients during the above mentioned period of these, 550 were admitted to the Intensive Care Unit within the Division. These are referred from maternity units in the Toronto area, the majority suffering from respiratory disease in association with low birth weight, the remainder having congenital heart disease metabolic disorders or neonatal surgical conditions (9) During this period hypoglycaemia was diagnosed in 52 infants. We found 13 infants with isolated symptomatic hypoglycaemia, of whom 6 had cardiomegaly in association with the hypoglycaemia None of the 13 infants had any of the following conditions respiratory distress syndrome transient tachypnoea of the newborn (?) congenital heart disease sepsis anaemia or polycythaemia (3) all of which may be associated with cardiac enlargement None of the infants had Apgar scores less than 5 and delivery was normal or by low forceps

Blood samples for sugar determination were obtained by heel prick or venous puncture on admission and thereafter at varying intervals depending on the infant s condition and the initial result The sampling was carried out every 2 to 6 hours until normal levels were attained

The blood sugar was measured by a modification of the method (8) using the reduction of potassium ferricyanide to potassium ferrocyanide by glucose

Hypoglycaemia was diagnosed when the blood sugar was reported as being <25 mg/100 ml in the first 24 hours of life and <30 mg/100 ml after that period

It is acknowledged that measurement of the cardiothoracic ratio on an AP film rather than PA film with the patient recumbent rather than upright, and at a focal film distance of 40 inches rather than 72 inches tends to vitiate the accuracy of this measurement as an absolute value However as the circumstances were the same in all cases, the cardiothoracic ratio (C/T) can be considered a valid way of estimating change in heart size The cardic enlargement was noted independently of the clinical findings, and diagnosed on comparison with a large volume of neonatal chest films.

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Table 1 *Symptoms and X ray findings of cases 1–6*

No	Sex	Gestation (week)	B W (g)	Age at onset of symptoms (hr)	Symptoms	X ray chest: Initial and final C/T ratio	X ray chest: Pulm oedema	Diagnosis
1	♂	39	2 300	5	Apnoea Jittery	61–51	+	Dysmaturity
2	♂	40	2 580	24	Tachypnoea Jittery	63–47	+	Dysmaturity
3	♂	39	2 180	7	Lethargy Cyanotic spells Tachycardia Tachypnoea	68–56	+	Dysmaturity
4	♂	36	1 450	36	Tachypnoea Jittery	66–55	+	Dysmaturity
5	♂	38	1 920	8	Tachypnoea Jittery	57–46	+	Dysmaturity
6	♀	36	1 900	46	Tachypnoea Jittery	62–51	+	Dysmaturity

RESULTS

There were 6 infants (Table 1) of the 52 with hypoglycaemia who also had radiographic cardiac enlargement. This ranged in severity from a marginally normal cardiothoracic ratio of 57% to a grossly abnormal 68% (Fig 1). All the cases also exhibited pulmonary oedema of varying degree, the most severe having a small pleural effusion. In addition to the classical signs of symptomatic hypoglycaemia, 5 of the 6 infants had tachypnoea (respiratory rate >65/min) and one, symptoms suggestive of congestive heart failure (Case 3) as manifest by tachypnoea, tachycardia, and hepatomegaly. No crepitations were heard over the lung fields, however, interstitial pulmonary oedema is notably lacking in this physical sign unless true alveolar flooding is present. In every case the clinical sings and symptoms disappeared on correction of the hypoglycaemia. There was no doubt at all that the heart originally large became smaller and the pulmonary oedema disappeared (Fig 2). The course of one of the infants (Case 3) is shown in Fig 3 *a* and *b* the films being taken at the age of 60 and 90 hours.

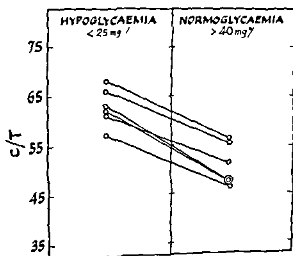


Fig 1 Cardiothoracic ratio (C/T)—Cases 1–6

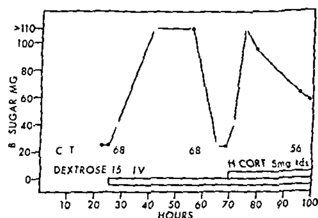


Fig 2 Case 3 demonstrating blood sugar levels and cardiothoracic ratio (C/T)

The 6 infants were small for gestational age using the criteria of Usher & McLean (12) Electrocardiographs were taken in Cases 1–4 and were within normal limits for newborn infants

As previously noted above there were 7 further infants who were comparably hypoglycaemic for approximately the same period of time but had no cardiac enlargement Six of these were small for gestational age and all had hypoglycaemic symptoms related to the central nervous system and received active therapy All 13 infants had similar prenatal natal and postnatal histories Their serum electrolytes and haematocrits were within normal limits (Table 2)

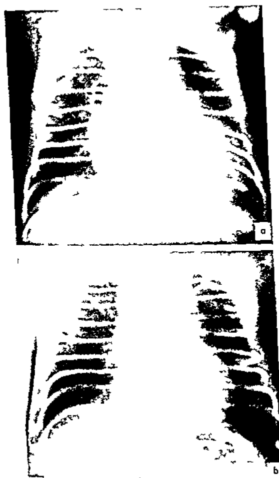


Fig 3 a and *b* X ray findings during and after period of hypoglycaemia in Case 3

Table 2 *Apgar score haematocrit method of delivery and ECG findings*

Case no	Apgar score	Haematocrit	Method of delivery[a]	ECG findings
1	6	58	N/D	Normal
2	9	66	L/F	Normal
3	5	62	L/F	Normal
4	7	60	N/D	Normal
5	5	59	M/F	Not done
6	10	60	N/D	Not done

N/D = Normal delivery
L/F = Low forceps
M/F = Mid forceps

DISCUSSION

Radiological (cardiac enlargement, pulmonary oedema) or clinical (tachypnoea tachycardia hepatomegaly) signs suggestive of cardiac failure resolving on treatment of hypoglycaemia have not previously been described

The incidence of symptoms in hypoglycaemia varies according to different authors (4 10) The majority of the symptoms relate to the central nervous system and vary from lethargy to convulsions

Since cardiac enlargement disappeared upon the attainment of normoglycaemia, we would suggest that the cause of the heart failure may be related to insufficient cardiac energy substrates the body content of both glycogen and lipid being deficient in dysmature infants (1 10)

Yao and co workers (13) have shown that small for date infants may have increased blood red cell and plasma volumes compared with other newborn infants We have no data on these values on our cases although the haematocrit levels were within normal limits It is therefore possible that some part of the cardiac failure has been due to circulatory overloading However the close correlation between the radiological and clinical findings and the blood sugar levels were still very striking

It is important to recognise this manifestation of hypoglycaemia in order that it can be

rapidly corrected, if necessary with corticosteroids (6) In this way the period during which the infant has signs and symptoms of central nervous system and cardiovascular system is minimised The condition should be considered in the differential diagnosis of cardiomegaly of heart failure in the newborn period

In the light of our finding of the deleterious effects on the cardiovascular system and of others suggesting the possibility of hypoglycaemic damage to the central nervous system (5), we cannot agree with the suggestion of Griffiths (7) based on the post mortem findings in 22 infants with hypoglycaemia, that treatment may be unnecessary

SUMMARY

Fifty two infants with hypoglycaemia were seen over a 12 month period Six of these infants had cardiac enlargement with signs of failure and pulmonary oedema in association with isolated hypoglycaemia Signs and symptoms suggestive of cardiac failure corresponded to the period of hypoglycaemia and disappeared when blood sugar levels were raised by appropriate treatment

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URINARY GLYCOSAMINOGLYCAN EXCRETION IN THE NEONATAL PERIOD

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An increase in urinary glycosaminoglycans (GAG) is a useful diagnostic feature of the hereditary mucopolysaccharidoses However there is little available information on the excretion of these substances in the early weeks of life when there is difficulty in obtaining 24 hour samples of urine

In this laboratory a cetylpyridinium chloride (CPC) turbidity test is used as a semiquantitative screening test for excess GAG excretion (4) Although this test has proved useful in older children (5) the lack of adequate information on the normal GAG excretion by neonates makes it difficult to interpret screening tests in such subjects The same applies to the CPC precipitable uronic acid which is usually measured when a screening test is positive Inspection of published data shows that only two of the normal children studied by Teller et al (6) and only five of those studied by Manley et al. (3) were less than three months old

In view of the difficulties concerning both 24 hour urine collections and the interpretation of uronic acid excretion this investigation was planned to determine the range of normal CPC precipitable uronic acid in random urine samples collected from infants under six months of age

MATERIALS AND METHODS

Random samples of urine were collected from 160 normal neonates (birth weight 3–4 kg gestation 37–42 weeks) while they were in the newborn nursery at Southmead Hospital Bristol or at various intervals after discharge from hospital A further 30 samples were collected from infants aged 3–6 months

The CPC precipitable GAG were isolated by the method of Di Ferrante (?) using reduced volumes where necessary and the uronic acid content was estimated by the method of Bitter & Muir (1) Urinary creatinine was estimated using an autoanalyser method (Technicon method file N 11)

RESULTS

The CPC precipitable uronic acid excretion expressed as mg per g of creatinine is shown in Fig 1 Results on six samples were excluded as the creatinine concentration was less than 5 mg per 100 ml and an error of only 1 mg either side of this figure would markedly affect the ratio The normal range is depicted by the lines drawn by inspection as there were insufficient samples at certain ages to adequately undertake a statistical analysis There was no apparent difference between males and females Thirty samples from infants aged three to six months gave a mean uronic acid result per g of creatinine of 20 5 ± 8 9 (S D)

DISCUSSION

The CPC citrate screening test (4) gives approximately 3 % positive results which require further investigation in children over one year of age (5) However if 300 CPC units per g creatinine is accepted as the upper limit of

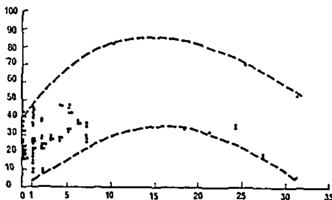


Fig 1 Showing uronic acid excretion per g of creatinine against age in days

normal up to six months of age, we find a large number of positive results in neonates When CPC precipitable uronic acid is estimated, many samples give a result above the normal upper limit of 32 mg per g creatinine at six months of age given by Teller et al (6) Our results in normal infants aged three to six months are in close agreement with theirs However, the results presented here show that the excretion of GAG as reflected by CPC precipitable uronic acid can be above this upper limit in neonates

Thus GAG excretion expressed per g creatinine is higher in the neonatal period than at any other time in life There is an increase from birth reaching a peak in the second week and thereafter declining throughout childhood to adult levels

We believe that a screening test which takes urine concentration into account is essential at this age since only random samples of urine are easily obtained In our screening test (4) we still retain an upper limit of normal at 300 CPC units/g creatinine for samples from infants below the age of six months We prefer to do uronic acid estimations on samples giving results above this because we would be less likely to miss a genuine positive result under these conditions and the additional work load of analysing false positives is not excessive

The early diagnosis of the mucopolysaccharidoses is essential if genetic counselling is to be part of the management of this group of disorders The fact that glycosaminoglycan excretion, expressed as CPC precipitable uronic acid per g of creatinine, is high in the neonatal period may make early diagnosis difficult at this time if reliance is placed solely on urinary excretion studies

SUMMARY

Urinary glycosaminoglycan excretion relative to creatinine is high during the neonatal period reaching a peak in the second week of life and thereafter declining throughout childhood The difficulty in early diagnosis of the mucopolysaccharidoses during the neonatal period is emphasized

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INHIBITION OF ERYTHROPOIESIS BY PLASMA FROM NEWBORN INFANTS

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Several investigators have previously stressed the marked and rapid change of erythropoiesis in the normal newborn infant During the first week of life the reticulocyte count decreases from high levels to normal or below normal adult levels (23 24) While the erythropoietin levels are slightly elevated in cord blood no erythropoietin is detectable in plasma from infants during the first weeks of life (9) The rapid decrease of erythropoiesis can be due to disappearance and/or utilization of erythropoietin alone but an inhibitory mechanism may also be involved (10) An analogous condition can be found in high altitude natives brought to sea level A decrease of erythropoiesis occurs and an inhibiting effect of plasma from such persons on erythropoiesis in recipient animals has been describ d (4 19 20 21) Several authors have reported the presenc of erythropoiesis inhibiting factors in plasma from hypertransfused animals as well (12 19 26 27) while others have b en unable to confirm this (3 16)

These findings raise the following questions Doe an inhibiting factor participate in the depression of erythropoiesis in the newborn infant? If so from which day of life and for how long time is this factor detectable? The present paper reports investigations to answer these questions

MATERIAL

Normal infants

Cord blood was collected from 7 infants born after normal pregnancies and with normal deliveries Blood (4-10 ml plasma) was further collected by venesection from 11 normal infants between 2 and 84 days of life Three of these infants had been admitted to the department because previous siblings had died during infancy but were themselves found to be healthy The remaining 8 sampl s were collected in the obstetrical department from infants born after normal pregnancies and deliveries. In addition three infants in the recovery period after non-surgical treatment for pyloric stenosis were also investigated

Hyperbilirubinemic infants

Because of the difficulties in obtaining from normal infants the large amount of plasma needed for the bioassays hyperbilirubinemic infants with normal hemoglobin levels were also investigated Twenty one of these infants were 2 and 3 days old The cause of the hyperbilirubinemia in these infants was ABO incompatibility Four of these infants were premature Hemoglobin levels were above 18 9 g ml and total bilirubin between 13 and 23 mg/100 ml The reticulocyte levels were within normal rance Coombs test was negative None of these infants were re admitted later because of prolonged jaundice

The clinical data on the 25 hyperbilirubinemic infants 4 to 6 days old are listed in Tables 2–4 These infarts had hemog obin levels above 18 2 g/100 ml and reticulocyte levels below 2 5% at the tim of blood sampling The Coombs test was negative in all infants In 10 infants the percentage of unconjugated bilirubin was in the range of 83 95% Blood was col ected during exchange transfusion In premature infants the first 20 ml of blood withdrawn was ampled and in mature infants the first 40 ml

Controls

Plasma from normal adults with normal hemoglobin was u ed for control samples To test the possibility that unconjugated bilirubin was influencing the erythropoiesis in the recipient mice bilirubin was added to the normal adult plasma in the following manner To 5 mmol EDTA+0 4% NaHCO was added 0 1 N NaOH until pH reached 11 40 mg bilirubin (Sigma) was added and mixed with citrated normal

Table 1 *Effect of plasma from normal newborn infants on ^{59}Fe incorporation into red blood cells (RBC) of hypoxia induced polycythemic mice stimulated with a second period of hypoxia of 8 hours*

Relevant clinical and laboratory data are listed The ^{59}Fe uptake values in all recipient mice in each group are pooled and the mean and S E M are shown The statistical significance of the difference between the plasma treated group and the saline controls is indicated by asterisks in this and subsequent tables (*** = $p<0\,001$ ** = $0\,001<p<0\,005$ * = $0\,005<p<0\,01$) Figures in parentheses indicate number of recipient mice

Name	Age (days)	Birth weight/length (g/cm)	Hemoglobin (g/100 ml)	Reticulocytes ( )	Percent ^{59}Fe uptake in RBC of recipient mice ±S E
G U A	4	3 250/51	20 7	1 0	8 2±0 3 (2) ***
G U N	4	2 930/49	21 0	0 8	8 8±1 2 (4) ***
A N D	4	2 780/49	21 0	1 1	9 5±0 9 (2) ***
K A M	5	2 520/48	20 7	1 7	8 8±0 1 (5) ***
B J E	6	3 120/51	20 8	0 4	7 9±1 1 (4) ***
Neonatal plasma pooled					8 6±0 4 (17) ***
Saline pooled					33 7±2 6 (26) —
Adult plasma pooled					26 1±2 0 (31) n s

adult plasma which was used in the exchange trans fusion and pH was adjusted to 7 5 with 0 1 N HCl (8) The bilirubin concentration in the plasma was de termined according to Jendrassik & Grof (11) In one assay different fractions from an exchange trans fusion were used to test the effect of dilution The first sample consisted of the first 40 ml of infant blood the second fraction the next 40 ml and the third fraction the third 40 ml blood drawn from a hyperbilirubinemic 3 day old infant

BIOASSAY

Randomized female mice of the NMRI/BOM strain weighing between 25 and 30 g were used The mice were made polycythemic by hypoxia in a low pres sure chamber at 0 4 atm for 3 weeks Following removal from the chamber the erythropoiesis de creased to zero On the fourth day out of chamber erythropoiesis was again stimulated by endogenous or exogenous erythropoietin Endogenous erythro poietin was produced by 6–11 hours of hypoxia (0 4 atm) The plasma to be tested and saline were given immediately before and after this second hypoxic period and twice on the following day As exogenous erythropoietin was used an internal laboratory stand ard of erythropoietin obtained by dialysis and lyo philization of urine from a patient with aplastic anemia The erythropoietin standard was injected sub cutaneously and simultaneously with the plasma to be tested or saline in doses of 0 1 mg at 9 a m and 4 p m on the fourth day and 0 2 mg at the same hours on the fifth day out of tank Altogether 0 6 mg of the internal standard was injected and this is equal to 0 15 units of the Standard Erythropoietin B in our assay The mice responded to these doses with approximately 10 Fe uptake in RBC In some as says a total dose of either 0 08 or 4 0 mg was in jected (Table 4) In all experiments the mice received 0 5 ml × 4 of untreated plasma or 0 4 ml × 4 of saline 0 1 μCi ^{59}Fe dissolved in 0 5 ml saline was injected intraperitoneally at 9 a m on the sixth ex hypoxic day and the mice were venipunctured by the femoral vein after ether anaesthesia 48 hours later A well scintillation counter was used (Frieseke & Hoepfner Erlangen Bruch Germany) The radio activity in 0 5 ml blood sample was measured with a gamma sensitive crystal with an efficiency of about 6 5 × 10^{4} cpm per 0 1 μCi of ^{137}Cs at a background of 440–470 cpm Mice with a hematocrit lower than 53 were excluded The inhibition was calculated from the difference in ^{59}Fe uptake between the control mice given saline and the groups of mice given plasma injections

RESULTS

Table 1 shows the effect of normal neonatal plasma on ^{59}Fe incorporation in the red blood cells (RBC) of plethoric mice stimulated with hypoxia Plasma withdrawn from normal new borns between the 4th and 6th days significantly reduced the ^{59}Fe incorporation in the red blood cells of the recipient mice compared with saline controls

Table 2 and 3 show the effect of plasma sampled during exchange transfusions per formed on the 4th 5th and 6th days of life

Table 2 depicts the results in mice in which the erythropoiesis was stimulated with 8 hours hypoxia and Table 3 in mice in which erythro poiesis was stimulated with injections of 0 6 mg of our internal erythropoietin standard In both types of recipients the ^{59}Fe incorporation

Table 2 *Effect of plasma from newborn hyperbilirubinemic infants on ^{59}Fe incorporation into red blood cells (RBC) of hypoxia induced polycythemic mice stimulated with a second period of hypoxia of 8 hours*

Relevant clinical and laboratory data are listed The ^{59}Fe uptake in all recipient mice in each group are pooled and the mean and S E.M are shown

Name	Age (days)	Birth weight/ length (g/cm)	Clinical comments	Hemoglobin (g/100 ml)	Reticulo- cytes (%)	Bilirubin (mg/100 ml)	Percent Fe uptake in RBC of recipient mice ±S E
L H S	4	1 600/40	Premature	18 2	1 2	17 4	3 6±0 4 (8) *
Y P E.	4	1 800/41	Premature	20 9	0 5	22.2	3.5±0 4 (5) ***
K. W E.	4	2 890/48		19 4	2.5	25 0	6 2±0 8 (5)
E. L. A	4	3 640/49	Diabetic mother	18 2	1 3	23 0	3 5±0 8 (7) *
M S K.	5	3 680/50		19 5	1 3	35 0	6 4±0 7 (6) **
K. E A	6	1 960/44	Premature	18 8	2 0	19 7	5 4±1 1 (7)
Neonatal plasma pooled							4 7±0 5 (38) **
Saline pooled							12 1±1 2 (33) —
Adult plasma pooled							15 4±1 9 (28) n s

was significantly reduced when the animals were injected with plasma from newborn infants aged 4–6 days compared with mice injected with saline or normal adult plasma

Table 4 illustrates the clinical and laboratory data of the 4–6-day-old infants not presented in Tables 1–3 The degree of the second erythropoietic stimulation is different from that in the assays presented in the previous tables

Table 5 shows the effect of bilirubin on erythropoiesis Plasma with added bilirubin increased the ^{9}Fe uptake however not significantly while the neonatal plasma with the same bilirubin content significantly reduced the uptake compared with the saline group

Plasma withdrawn between the age of 4–6 days of life from premature and mature infants from normal and hyperbilirubinemic infants significantly inhibited the ^{9}Fe incorporation in the RBC of the recipient mice Since there is good agreement between these results it has been found justified to visualize all the data in one figure In Fig 1 is included the data from all the assays performed with plasma from infants irrespective of the clinical condition of the donor infant and the type of sec

Table 3 *Effect of plasma from newborn hyperbilirubinemic infants on ^{59}Fe incorporation into red blood cells (RBC) of hypoxia induced polycythemic mice stimulated with 0 6 mg of an internal laboratory standard of erythropoietin*

Relevant clinical and laboratory data are listed The Fe uptake in all recipient mice in each group are pooled and the mean and S E M are shown

Name	Age (days)	Birth weight/ length (g/cm)	Clinical comments	Hemoglobin (g/100 ml)	Reticulo- cytes ( )	Bilirubin (mg/100 ml)	Percent Fe uptake in RBC of recipient mice ±S E.
U G R	4	3 210/47	ABO incompatible	20 8	1 3	21 6	2 0±0 2 (5)
J E A	4	2 3 0 47	Premature	20 9	1 4	23 0	1 7±0 3 (6) *
E L A	4	3 640 49	Diabetic mother	18 2	1 3	23 0	2.7±0 5 (5) ***
T V E	5	3 430/49		20 9	1.2	29 5	2 0±0 5 (5)
P L Y	6	1 960 43	Premature	20 9	1 2	21 0	2.2±0 2 (5)
S C H	6	3 025/50		21 7	1 7	21 0	2.1±0 2 (6) ***
Neonatal plasma pooled							2.0±0 2 (33)
Saline pooled							9 6±0 7 (23)
Adult plasma pooled							9 3±1.2 (20)

Table 1 *Effect of plasma from normal newborn infants on ^{59}Fe incorporation into red blood cells (RBC) of hypoxia induced polycythemic mice stimulated with a second period of hypoxia of 8 hours*

Relevant clinical and laboratory data are listed The ^{59}Fe uptake values in all recipient mice in each group are pooled and the mean and S E M are shown The statistical significance of the difference between the plasma treated group and the saline controls is indicated by asterisks in this and subsequent tables (*** = $p<0.001$ ** = $0.001<p<0.005$ * = $0.005<p<0.01$) Figures in parentheses indicate number of recipient mice

Name	Age (days)	Birth weight/length (g/cm)	Hemoglobin (g/100 ml)	Reticulocytes (%)	Percent ^{59}Fe uptake in RBC of recipient mice ±S E
G U A	4	3 250/51	20 7	1 0	8 2±0 3 (2) ***
G U N	4	2 930/49	21 0	0 8	8 8±1 2 (4) ***
A N D	4	2 780/49	21 0	1 1	9 5±0 9 (2) ***
K A M	5	2 520/48	20 7	1 7	8 8±0 1 (5) ***
B J E	6	3 120/51	20 8	0 4	7 9±1 1 (4) ***
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RESULTS

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Table 2 and 3 show the effect of plasma sampled during exchange transfusions performed on the 4th 5th and 6th days of life

Table 2 depicts the results in mice in which the erythropoiesis was stimulated with 8 hours hypoxia and Table 3 in mice in which erythropoiesis was stimulated with injections of 0 6 mg of our internal erythropoietin standard In both types of recipients the ^{59}Fe incorporation

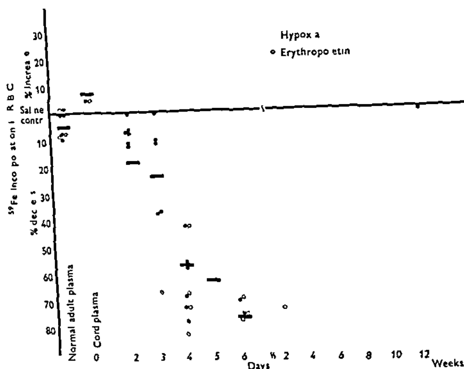


Fig. 1. Effect of neonatal plasma on ^{59}Fe uptake in RBC of polycythemic mice in which erythropoiesis has been stimulated with endogenous (hypoxia) or exogenous erythropoietin. The baseline illustrates ^{59}Fe uptake levels of saline injected mice. Circles or points represent the mean of each plasma injected group expressed as percentage increase or decrase of ^{59}Fe uptake in relation to saline control levels. — Mean of pooled data in each type of plasma. Age of infants at time of sampling is indicated at the baseline.

poiesis was stimulated by hypoxia or exogenous erythropoietin. The neonatal plasma also inhibited the reticulocyte response to hypoxia in mice. The latter data will be reported elsewhere (25).

The results are in contrast with those of Matoth & Zaizov (16). These authors could not demonstrate any inhibitory effect of pooled plasma withdrawn from 6-day-old infants on the ^{59}Fe uptake in red blood cells of normal mice which were stimulated by 18 hours hypoxia. Neither did neonatal plasma inhibit the erythropoietic effect of plasma from an anemic thalassemia patient, using hypertransfused mice as recipient animals (16).

Several methods for determination of erythropoiesis inhibiting factors have been described and are discussed elsewhere (25, 28). The present authors are of the opinion that the post hypoxic polycythemic mice are the most sensitive recipient animals in erythropoiesis inhibition studies. From our data it also seems important that the inhibiting factor is injected into the recipient animals shortly before or at the same time as the stimulator of erythropoiesis is applied. The differences between our results and the results of Matoth & Zaizov may possibly be explained by the differences in the assay systems.

Because of the restricted amount of blood that can be obtained from healthy newborn infants, plasma was also drawn from hyperbilirubinemic infants during exchange transfusions. Both normal and jaundiced infants had normal hemoglobin levels and showed evidence of the normal suppression of erythropoiesis after birth. A low percentage of conjugated bilirubin indicated that the cause of the hyperbilirubinemia was immature liver function and not excessive hemolysis. Bilirubin has previ

Table 4 *Relevant clinical and laboratory data of infants between 4 and 84 days of age*

The percentage inhibition of ^{59}Fe incorporation into RBC of hypoxia induced polycythemic mice stimulated with hypoxia or with an internal laboratory standard of erythropoietin is indicated The mean of the percentage of inhibition and S E M for each infant group are given The statistical significance of the difference between infant plasma injected mice and saline injected mice is indicated by asterisks (see Table 1)

Name	Age (days)	Birth weight/ length (g/cm)	Clinical comments	Hemoglobin (g/100 ml)	Reticulocytes ( )	Bilirubin (mg/100 ml)	Content (unconjug)	Percentage inhibition ±S E of ^{59}Fe uptake	Injected mice *n*
C L A	4	2 320/46	Premature	18 0	1 1	18 2	94	36± 2 7	6 **
P A F	4	1 540/41	Prem ABO incomp	20 8	1 9	21 4	90	22± 3 8	5 *
E N O	4	3 350/52	ABO incompatibility	19 3	0 4	21 4	83	73± 4 4	6 ***
R U D	4	1 800/42	Diabetic mother	18 8	2 0	20 5	—	42± 5 2	4 **
S K O	4	3 495/41	Hyperbili	18 8	2 0	18 8	95	83± 5 5	6 ***
T R L	5	3 100/49	AO incomp	19 3	2 0	22 6	—	55± 4 0	6 ***
T T H	5	1 738/38	AO incomp prem	20 8	1 5	25 0	89	56± 6 0	6 ***
H M N	6	1 830/44	Prem	20 8	0 4	18 7	88	76± 4 6	6 ***
L I S	6	3 800/52	BO incomp	18 8	1 2	11 0	—	74± 3 2	4 ***
G A A	6	3 185/52	Hyperbili forceps	18 0	2 0	13 9	—	83± 6 1	4 ***
H O F	13	3 250/51	Normal	18 8	1 0	1 3	—	72± 4 4	4 ***
S Y F	14	3 600/53	Normal	16 1	0 6	—	—	68±12 0	5 **
N A N	28	2 940/50	Normal	16 5	0 5	—	—	38±14 2	4 *
K O R	50	3 600/53	Pyloric stenosis	13 2	0 7	—	—	40± 9 2	5 **
G O L	56	3 980/51	Pyloric stenosis	13 5	0 6	—	—	22±10 1	5 n s
S K U	70	3 200/51	Pyloric stenosis	12 6	2 1	—	—	34± 9 0	5 *
S T E	84	3 850/53	Normal	12 0	4 2	—	—	3± 4 0	5 n s

ondary stimulation of erythropoiesis in the recipient mice Fig 1 shows that plasma from normal adults reduced the ^{59}Fe uptake slightly but insignificantly in the recipient mice Mice injected with plasma from cord blood had a higher ^{59}Fe uptake than the mice injected with saline but the difference was not significant Mice injected with plasma withdrawn from infants aged 2 days to 6 weeks had a lower uptake than the controls and the differences were significant from the 4th to the 14th day of life From the age of 6–12 weeks no significant inhibitory effect of the plasma could any longer be demonstrated Plasma obtained at the 2nd and 3rd days of life inhibited the ^{59}Fe uptake however insignificantly

Table 5 *Effect of bilirubin added to normal adult plasma on ^{59}Fe incorporation into red blood cells (RBC) of hypoxia induced polycythemic mice stimulated with a second period of hypoxia of 6 hours*

The bilirubin content of the injected material is given

Injected material	Total bilirubin content (mg/100 ml)	Percent ^{59}Fe uptake in RBC of recipient mice ±S E
Adult plasma	1 2	7 2±0 8 (5) n s
Adult plasma with added bilirubin	25 0	11 3±1 3 (5) n s
Neonatal plasma	22 2	3 5±0 4 (5) ***
Saline	—	7 8±0 3 (8) —

When plasma from a 3 day old infant withdrawn during an exchange transfusion was separated in portions of 40 ml one could demonstrate a diluting effect on the inhibitory influence Fig 2 illustrates the ^{59}Fe uptake in the recipient mice after injections with the different fractions compared with the effect of the donor plasma in the actual transfusion and with saline Plasma from the first 40 ml and the second 40 ml had a significant inhibitory effect on the ^{59}Fe uptake of the red blood cells in the polycythemic mice stimulated with endogenous erythropoietin

DISCUSSION

Plasma from normal and hyperbilirubinemic newborn infants inhibited ^{59}Fe incorporation in RBC of polycythemic mice in which erythro-

and 12 weeks of age did not consistently show any inhibitory effect In this age group the hemoglobin concentration falls to its lowest level after birth Halvorsen (9) was unable to demonstrate any erythropoietin in plasma from infants below 54 days of life but the assay is not sensitive enough to differentiate between normal and low normal erythropoietin levels At present one cannot therefore state whether the reduction of inhibitory activity is due to disappearance of the inhibitor or an increase in plasma erythropoietin which counteracts the inhibitor

The physiological role of inhibitors in erythropoiesis is still debated (3 16) and it is so far unknown whether the inhibitors are constantly present in plasma or urine or produced as a response to a surplus of oxygen available in the tissues The present data do not provide evidence in any of these directions but studies on urine samples after separation of erythropoietin from the inhibitor may give the answer to this question (15)

The cellular growth in epidermis is regulated by tissue specific inhibitors of mitosis termed chalones by Bullough (2) Rytomaa & Kiviniemi (22) have extracted inhibitors of myelopoiesis and erythropoiesis from mature granulocytes and erythrocytes and they have also been able to demonstrate such substances in serum The relationship between the inhibitors in plasma of newborn infants and the erythrocytic chalone can only be speculated upon at the present time However it is reasonable to suggest that the erythropoietic inhibitors may be related to chalones and that they are of physiological importance In the neonatal period such inhibitors may effectively depress erythropoiesis and the hematological changes following birth may be explained as a result of variations in the stimulators and inhibitors of erythropoiesis

SUMMARY

The marked and rapid decrease of erythropoiesis in the normal newborn infants may be due to disappearance of erythropoietin alone but an inhibitory mechanism may also be involved Several authors have previously reported the presence of erythropoiesis inhibiting factors in plasma from high altitude natives brought to sea level and in plasma from hypertransfused animals The present studies of ⁵⁹Fe incorporation in RBC of polycythemic mice injected with plasma from normal and hyperbilirubinemic newborn infants show that erythropoietic inhibitor(s) is present in the plasma of infants at least from the fourth day of life This inhibition was not due to the bilirubin content. A possible influence of estrogen was discussed and found to be unlikely The relation to chalones the tissue specific inhibitors of mitosis is discussed.

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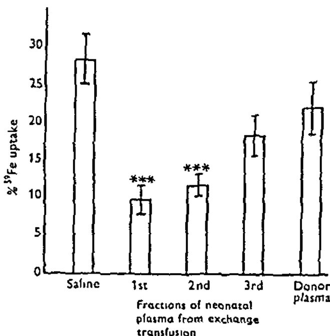


Fig 2 Effect of plasma from a 3 day old hyperbilirubinemic infant withdrawn during the first part of an exchange transfusion on 59Fe uptake in RBC of polycythemic mice in which erythropoiesis has been stimulated with endogenous erythropoietin (hypoxia) The statistical significance of the difference between the plasma injected group and saline controls is indicated by asterisks (*** = $p<0.001$) Columns represent the mean of each assay group ±S E

ously been reported to have an erythropoiesis stimulating effect (1) In our assay there was no statistically significant difference in the 59Fe uptake in RBC of mice injected with normal adult plasma, with or without added bilirubin

The reduced 59Fe uptake in the RBC of the recipient mice following plasma injections may be caused by different mechanisms A reduced iron incorporation into heme or a specific inhibition of the erythropoietic stimulating effect of erythropoietin are the two most likely mechanisms

Based on analysis of serum iron in the injected plasmas and simultaneous 59Fe uptake and reticulocyte studies in the recipient mice (25), the present authors favour the interpretation that the reduction in 59Fe uptake is due to specific inhibitors of erythropoiesis This is in accordance with the interpretations of the findings of proteins which reduce 59Fe uptake in recipient animals (7, 14, 15, 19) At which cellular level this inhibition occurs, is, however, unclear

An inhibitory effect of estrogens on erythropoiesis has been demonstrated previously (17) Since the estrogen content in newborn plasma is reported to be high (18) the inhibitory effect could be explained by high estrogen levels The level is, however, higher in cord plasma than later in the first week, which does not fit with our findings that the inhibition is most marked from the fourth day of life (18) The doses of estrogen used in the inhibition studies were much higher than the possible content in 2 ml infant plasma It is therefore unlikely that the inhibitory effect in our experiments could be an estrogen effect.

The second question to be commented upon is the time of appearance of the inhibitor in infant plasma after birth In plasma from high altitude natives brought to sea level, Reynafarje could demonstrate an inhibitory effect on 59Fe uptake after 72 hours and an optimal effect on the 7th–9th days after transfer to sea level (21) Whitcomb & Moore reported that the inhibitory effect of sheep plasma could not consistently be demonstrated until 72 hours following hypertransfusion (28)

In our present experiments plasma drawn from 2 day-old infants exhibited an inhibitory effect on the 59Fe uptake The pooled means, however were not statistically significant in infants below 4 days of age Plasma from older infants caused a 70% reduction of iron uptake compared to the saline controls Halvorsen and Finne have previously demonstrated high levels of erythropoietin in cord blood (6 9) These findings are consistent with the concept that the fetus produces erythropoietin as a response to hypoxia (5 6 24) Following birth the erythropoietin levels decrease to not detectable levels (9) and it is assumed that the erythropoietin production stops The lag period before erythropoietic inhibitors are demonstrable in plasma may be the result of the time necessary for erythropoietin to disappear from plasma

Plasma withdrawn from infants between 6

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FAILURE TO THRIVE IN LEBANON I EXPERIENCE WITH SOME SIMPLE SOMATIC MEASUREMENTS

ABDALLAH A KANAWATI DONALD S McLAREN and ISABELLE ABU JAWDEH

From Nutrition Research Program School of Medicine American University of Beirut Beirut Lebanon

Some years ago an exploratory study of the socio-economic background of marasmus in Lebanon was undertaken by following over the course of 1 year nearly 200 consecutive cases after discharge from hospital (19) By the end of this period about 15 from the city of Beirut and 30 from the south of Lebanon had died and of the survivors more than 50 % were below the 3rd percentile (Boston) for weight Since then we have investigated many aspects of the problem of protein-calorie mal nutrition (P C M) and some of our findings have been summarised recently (22) Experi ence in Jordan and Lebanon led to the devel opment of a concept of the evolution of marasmus and kwashiorkor under different ecologic conditions (23) and a simple Scoring System by which to classify the various forms of severe P C M was devised (24) This has been applied over a period of 12 months in a hospital in Amman Jordan together with the collection of data on ecologic factors that might be related to undernutrition (18 25)

At the same time we have been studying the nature of the submerged" subclinical part of the P-C M iceberg from which arise the severe cases seen in hospital Several studies with a rather similar objective have been pub lished from other countries (1–4 10 12 13 20 26–28 30 33) Although circumstances vary considerably from one place to another and care must be exercised when comparisons are made we believe that our results are of interest to those concerned with the health of the pre school child in other developing coun tries Our primary intention in this paper has been to devise and test simple tools for the study of failure to thrive in the field rather than to investigate a representative population sample and obtain data on prevalence and in cidence In a later paper we intend to present the results we have obtained when the method devised and described here was applied in the field

MATERIAL AND METHODS

Th subjects in this study were Arab children be tween the ages of 3 and 48 months of Moslem Shii or Suni families who attended a clinic in one of the three areas of Lebanon These areas were 1) the village of Jba a, in the south of th country 2) Bourj al Barajne a suburb of Beirut and 3) Basta, an area in down town Beirut The groups for study were chosen serially from those children who were attend ing the clinic for immunization or for trivial illness Those with serious illnesses were excluded The en tire study was carried out by the same Arab physi cian (A. K.) assisted by the same Arab public health nurse (I A J) and took 12 months to complete (December 1967 to November 1968)

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Table 3 *Four somatic measurements expressed as percentages of standards according to season of observation*

	standards (mean ± S D)			
	Spring 239 cases	Summer 242 cases	Autumn 283 cases	Winter 467 cases
Weight	91.0 ± 13.4	89.0 ± 12.8	91.0 ± 14.5	91.0 ± 13.7
Height[b]	97.1 ± 4.8	97.6 ± 5.0	98.0 ± 5.0	97.1 ± 5.7
Head circumference	96.5 ± 4.6	96.2 ± 3.6	96.2 ± 3.3	96.1 ± 3.4
Mid arm circumference	92.0 ± 4.3	89.9 ± 8.1	89.6 ± 8.6	89.9 ± 8.4

[a] $0.02 > p > 0.01$ Summer v rest of year
[b] $0.05 > p > 0.02$ Autumn v rest of year
[c] $p < 0.001$ *Spring* v rest of year

and mid arm circumference did not differ significantly in the two sexes

Table 2 shows the same data divided according to location Only for height were there significant differences between the three areas

In Lebanon the year may be divided from the climatic point of view into four rather distinct seasons each of 3 months duration spring: March–May summer June–August autumn *September–November* and winter December–February Table 3 shows the data compared with regard to the season when they were collected Weight was significantly less in summer than in each of the other seasons height was significantly greater in autumn than in any of the other seasons head circumference differed *not according to season and mid arm* circumference was highly significantly greater in spring than in any of the other seasons

In Table 4 a similar comparison is made for season of birth In winter weight was significantly *less than in spring and height* less than in the other seasons

The correlations between the four somatic measurements are shown in Table 5 The correlations are much higher for the actual values of the measurements than the measurements expressed as percentage of standard

The four somatic measurements were used to classify the subjects according to the growth *they had achieved* in comparison with the international standards Several systems were applied The results obtained were almost identical but certain advantages were gained with each modification Initially an Index of Thriving employing all four somatic measurements in relation to international standards was used (17) This is quite satisfactory as a research tool but for widespread routine use it *has the disadvantage of requiring a weighing* machine an expensive delicate instrument often not used properly by semi skilled personnel The first refinement consisted of the head circumference and the mid arm circumference *in relation to international standards* The measurements are simple to make and require

Table 4 *Four somatic measurements expressed as percentages of standards according to season of birth*

	standards (mean ± S D)			
	Spring 94 cases	Summer [illegible]61 cases	Autumn 268 cases	Winter 3[illegible]0 cases
Weight	92.0 ± 13.5	90.6 ± 18.5	90.5 ± 14.9	89.3 ± 12.1
Height[a]	97.6 ± 5.1	97.9 ± 5.2	98.0 ± 5.7	96.9 ± 4.8
Head circumference	96.3 ± 5.4	96.4 ± 3.8	96.3 ± 3.7	96.2 ± 3.6
Mid arm circumference	91.0 ± 8.1	89.7 ± 8.5	89.5 ± 8.7	90.7 ± 7.9

[a] $0.01 > p > 0.001$ Winter v spring.
[b] $0.02 > p > 0.01$ *Winter v rest of year*

Table 1 *Four somatic measurements expressed as percentage of standards, in males and females*

	standards (mean ± S D)		
	Males 636 cases	Females 595 cas s	p
Weight	93 4 (±13 5)	87 6 (±13 2)	<0 001
Height	98 0 (±5 0)	96 8 (±5 9)	<0 001
Head circum ference	96 2 (±3 6)	96 2 (±3 7)	N S[a]
Mid arm cir cumference	90 5 (±8 1)	89 9 (±8 6)	N S

[a] N S = Not significant

Moslem year and local occurrences to check the age of the child to the nearest month Four somatic measurements weight height head circumference and mid arm circumference were made according to the procedure recommended by WHO (14) The pre sence or absence of oedema hepatomegaly derma tosis and hair changes of protein calorie malnutrition according to the descriptions of WHO (32) were noted

RESULTS

The sample comprised 1 231 children, 636 males and 595 females The largest number was seen in Bourj al Barajne (541) where the most active clinic is to be found There were 386 in Basta and 304 in Jba'a The propor tional distribution by sex and age was similar in the three locations It was therefore con sidered in order to pool data from all three locations for certain comparisons

Figs 1 and 2 show for males and females separately, mean values for the four somatic measurements expressed in relation to inter national standards (Boston standards for weight, height, and head circumference (29) and Warsaw (31) for mid arm circumference)

The curves for the four measurements were similar for the two sexes but there were some differences For boys and girls height and head circumference tended to be close together and relatively less affected than weight and mid arm circumference which also tended to re main close together There was a steady falling off in head circumference in both sexes from the age of 3 months with the value being ap proximately 95% Boston for most of the pe riod under study The height in both sexes tended to be nearer to the Boston standard than the head circumference during the first 12 months and for much of this time was equivalent to 100% Boston In the second year it fell below 95% Boston and during the 3rd and 4th years showed a greater deficit in both sexes than did head circumference

Weight in the male was nearly 100% Boston at 3 months and at or above this level until 6 months, after which it fell drastically to about 85% by 12 months The same down ward trend occurred in girls but at a lower level In both sexes there was thereafter a slow and fairly steady increase of weight percentage until 4 years The mid arm circumference in the female followed the weight rather closely in the first year In the male it reached the same low level then but without any early rise as was the case for weight During the 2nd to 4th years there was a steady increase in mid arm circumference in both sexes

The data for the four somatic measurements, expressed as percentages of the international standards, are compared for males and females of the entire sample in Table 1 For both sexes weight and mid arm circumference fell further short of the standards than did height and head circumference The percentage weight and height of girls were highly significantly lower than those of boys The head circumference

Table 2 *Four somatic measurements expressed as percentages of standards in three locations*

	standards (mean ± S D)		
	Basta 386 cases	Bourj al Barajne 541 cases	Jba a 304 cases
Weight	90 1 (±13 9)	91 2 (±13 9)	90 0 (±13 0)
Height[a]	98 0 (±5 1)	97 3 (±5 0)	96 9 (±5 4)
Head circum ference	96 0 (±3 4)	96 3 (±4 1)	96 2 (±3 2)
Mid arm circum ference	90 1 (±7 2)	90 2 (±8 3)	90 5 (±8 2)

[a] 0 02 > p > 0 01 Basta v Jba a
0 05 > p > 0 02 Basta v Bourj al Barajne

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[a] $0.01 > p > 0.001$ Winter v spring.
[b] $0.0[illegible] > p > 0.01$ Winter v rest of year

Table 5 *Correlation coefficients (r) for four somatic measurements expressed as actual values and as percentages of standards*

	r using actual values	*r* using percentages of standards
Weight v Height	0 943	0 671
Weight v Head circumference	0 857	0 558
Weight v Mid arm circumference	0 787	0 717
Height v Head circumference	0 843	0 445
Height v Mid arm circumference	0 650	0 305
Head circumference v Mid arm circumference	0 659	0 408

only a stout tape measure However, age needs to be known It was subsequently found that the ratio of these two measurements between 3 months and 4 years is constant for healthy children of both sexes It correlates highly with the Index of Thriving (19)

Thus, by any one of several means it is possible to compare the growth of pre school children Using the Index of Thriving two contrasting groups were identified those growing well as a control group, and those growing poorly as a failure to thrive group Detailed studies of environmental factors operating in these two groups and a long term follow up and evaluation of the methods used form the substance of subsequent reports

DISCUSSION

Assessment of failure to thrive

The need has long been recognized for a simple, objective means which can be used by general public health workers with a limited knowledge of nutrition to assess the physical development of population groups especially pre school children, in relation to their nutritional status

Gomez et al (6) first advocated the division of malnutrition (P C M) into 1st, 2nd and 3rd degrees according to the percentage deviation of the weight from the mean of a local standard group This classification has been widely adopted and has proved of great value in enabling results to be compared from different places However, standards established under differing circumstances of place, time methodology and population examined are bound to differ Reference to a widely recognized international" standard will assist in bringing uniformity of comparison but it assumes that healthy child populations everywhere measure the same This is not true if inborn effects of retardation in previous generations are present

There is evidence that healthy Lebanese children of a high socio economic class have weights and heights similar to those of European children (11) For routine use under field conditions weight has certain disadvantages A weighing machine is a delicate instrument, requiring intelligent use for reliable results The age must be known to the nearest month or so

For research purposes it seemed to us desirable to employ several measurements Body weight includes all compartments of the body and gives no indication of disproportionate changes in the size of different compartments Continuing increase in weight may bring a false sense of security when accumulation of water is taking place in early protein deficiency Conversely considerable falling off in weight gain velocity or actual loss of weight may occur over a short period of time in association with a brief but severe illness Height indicates the combined length of certain parts of the skeleton a part of the body relatively little affected by malnutrition Height cannot decrease absolutely Head circumference is made up mainly of external skull circumference but also includes small contributions from skin, subcutaneous tissue and muscle It correlates closely with brain size and at the lowest ranges with mental function Being mainly a skeletal measurement it behaves like height Mid arm circumference includes bone muscle fat subcutaneous tissue and skin As might be expected from its multi tissue nature it behaves much like body weight Other somatic meas

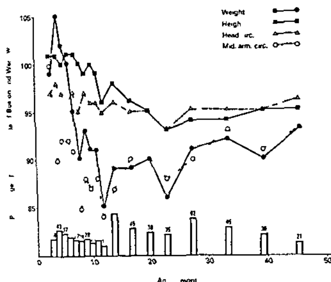


Fig 1 Four somatic measurements ex pressed as percentages of standards (see text) in relation to age Numb rs in each age group at foot of graph Data for boys.

urements some singly and others as ratios have been proposed but these also have various disadvantages including their age—dependence (16) Certain laboratory tests involve the ad ditional difficulties of collection of samples and their processing

We have studied each of these approaches to the problem and as indicated in the results pre ented here have come by way of the use of four somatic measurements in the Index of Thriving finally to select two of these for routine field use They are mid arm circum ference and head circumference expressed as a ratio which as we have shown elsewhere (19) appears to have none of the above men tioned disadvantages

Growth pattern of pre school children

The growth pattern as judged by the four somatic measurements shown in Figs 1 and 2 approximates to that of children in many de veloping countries (2 4 10 12–14 20 26

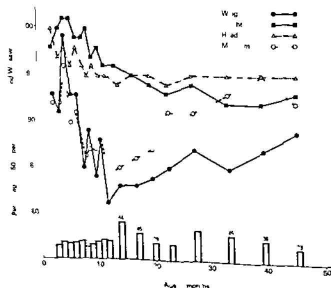


Fig 2 As Fig 1 Data for girls.

27, 29, 33) The gains in height and weight are satisfactory for the first 3–6 months of life After the sixth month weight falls to about 85% by the 12th month Similar declines were reported but to differing degrees from 67% (30) to 82% (1, 10) and over different periods of time 3 to 12 months (1, 10, 12) 3 to 18 months (2, 13, 20), or 3 to 36 months (30) In the second year a slow and steady increase of weight as percentage of standard takes place until 4 years Weight gain is actually above that achieved by the standard group A similar recovery has been reported in several countries but initiated at different ages and to varying degrees (1, 2, 10, 12, 13 19, 26, 29) The weight gains achieved by infants in the early months of life in several developing countries have been noted to be greater than by those in Europe and America (4) This was also observed in the present study but the other three measurements behaved differently This is further evidence that changes in weight do not reflect changes in different parts of the body The mid arm circumference, in general, follows the weight rather closely Height and head circumference show less deviation from the standards than do the other two measurements but having fallen they remain at the level of about 95% of standard until at least the 48th month

The results in Figs 1 and 2 and Table 5 show a high correlation between weight and mid arm circumference and between height and head circumference Thus the members of each of these pairs of measurements have similar growth rates It can be seen that skeletal measurements reflect better the long term effect of poor growth than do weight and mid arm circumference and that any declines in height and head circumference are irreversible changes Consequently such ratios as weight/height are not ideal measures because recovery in weight exceeds that in height and retardation of growth may be masked in this way

In this study females tended to be lighter than males in comparison with the standards for their own sex (Table 1) The difference between means of height and weight of the two sexes was highly significant ($P<0\ 001$) Evidence that cultural influences may favour boys has been reported in some parts of the world (9, 25, 33)

The heights, but not the other measurements, differed according to location (Table 2) The three communities are of the same ethnic stock and all are of a low socio economic background Further investigation of this point is indicated

The various differences in the measurements according to season of measurement and season of birth are rather complex and do not always have a ready explanation (Tables 3 and 4) The lower weight in summer than in other seasons may be attributable to the peak of diarrhoeal disease at this time and possibly also to the effect of high temperature and humidity on food intake There is similar evidence from many other countries (7, 15) Infants born in winter might be expected to be particularly exposed to the hazards of weaning during the summer months (7)

SUMMARY

During a period of 12 months 1 231 Lebanese Arab children of low socio economic class, 3–48 months of age living in three locations were measured The measurements were weight, height, head circumference and mid arm circumference The results were expressed as percentage of international standards for boys and girls separately The patterns of deviation from the standard values varied according to the measurement In general for both sexes there was a falling off between 6 and 18 months greatest for weight and mid arm circumference with some recovery later and less for height and head circumference without recovery later The possible influences of location and season of observation and birth were studied Using these four somatic measurements an Index of Thriving was developed for the subsequent study of contrasting groups of control and failure to thrive children The use of this

and other simple means of assessing failure to thrive in the field is discussed

ACKNOWLEDGEMENTS

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27, 29, 33) The gains in height and weight are satisfactory for the first 3–6 months of life After the sixth month weight falls to about 85% by the 12th month Similar declines were reported but to differing degrees from 67% (30) to 82% (1, 10) and over different periods of time 3 to 12 months (1, 10, 12), 3 to 18 months (2, 13, 20), or 3 to 36 months (30) In the second year a slow and steady increase of weight as percentage of standard takes place until 4 years Weight gain is actually above that achieved by the standard group A similar recovery has been reported in several countries but initiated at different ages and to varying degrees (1, 2, 10, 12, 13, 19, 26, 29) The weight gains achieved by infants in the early months of life in several developing countries have been noted to be greater than by those in Europe and America (4) This was also observed in the present study but the other three measurements behaved differently This is further evidence that changes in weight do not reflect changes in different parts of the body The mid arm circumference in general, follows the weight rather closely Height and head circumference show less deviation from the standards than do the other two measurements but having fallen they remain at the level of about 95% of standard until at least the 48th month

The results in Figs 1 and 2 and Table 5 show a high correlation between weight and mid arm circumference and between height and head circumference Thus the members of each of these pairs of measurements have similar growth rates It can be seen that skeletal measurements reflect better the long term effect of poor growth than do weight and mid-arm circumference and that any declines in height and head circumference are irreversible changes Consequently such ratios as weight/height are not ideal measures, because recovery in weight exceeds that in height and retardation of growth may be masked in this way

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POSTNATAL CHANGES IN SOME RED CELL PARAMETERS

Y MATOTH R ZAIZOV and I VARSANO

From the Department of Pediatrics Tel Aviv University Medical School and Beilinson Hospital Petah Tikva Israel

The red cell count is a basic study parameter of postnatal erythropoiesis Yet available data are derived mostly from work done before the advent of electronic counters Because of the error inherent in red cell counts done in the counting chamber the results were subject to considerable variation Indeed earlier workers have come to rely mainly on the hemoglobin mass in estimating the magnitude and timing of postnatal changes in the erythron However changes in the number of circulating red cells reflect the interplay between red cell production and destruction more accurately than do changes in the hemoglobin mass

In the present communication some current concepts of the dynamics of erythropoiesis after birth have been reevaluated in the light of more precise data on the red cell count and a study of some other red cell parameters

The data on the red cell count represent useful reference material for normal values in the age group 1 day to 12 weeks and are therefore reported in detail along with data on the hematocrit hemoglobin concentration and reticulocyte counts which have been obtained concurrently Other red cell parameters studied included red cell size and cell density distributions The latter is a good reflection of red cell age distribution (3)

MATERIAL AND METHODS

The red cell count, hemoglobin concentration hematocrit reticulocyte count and red cell size distribution were followed from birth to 12 weeks in an apparently healthy full term infant population delivered in this center Observations were made daily during the first week of life and at weekly intervals thereafter Between 10 and 20 observations were made for each day or week It is the practice in this hospital to clamp the cord early usually within one minute after the infant is delivered.

All counts (including the first day) were done on heel prick blood The skin was pricked with a Medi point blood lancet 3 mm deep and free flowing blood was aspirated Red cell counts and cell size distribution curves were determined in an electronic counter Hemoglobin was determined as oxyhemoglobin in a photoelectric colorimeter calibrated with a cyanmethemoglobin standard The hematocrit was determined as microhematocrit.

The total number of circulating red cells was calculated from the mean red cell count the mean weight for each group and a factor representing blood volume/kg body weight as determined by other workers (10 13 17) The figures used were 89 87 85 80 and 75 ml/kg for birth 1 week 2–4 weeks 5 weeks and 6–12 weeks respectively

Red cell density distribution was determined according to Danon & Marikovsky (3) Blood was drawn into a series of 20 microhematocrit capillary tubes containing mixtures of phthalate esters[a] with specific gravity increasing in steps of 0 04 from 1 036 to 1 138 Following centrifugation a separation of the cells heavier that the phthalate mixture in each tube was achieved

Price Jones curves were determined on the heavy and light fractions each representing 2–6% of the red cell population contained in the 2–5 extreme fractions Macrocytes having a volume of 186 μ and above have been excluded from calculations since this fraction was found to contain also large numbers of white cells and fat particles The mean corpuscular volume in various fractions (whole blood heavy and light fraction) was calculated from the weighted means in each cell volume step

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Table 1 *Red cell values in the first 12 weeks of life*

Age	No of cases	Hb gm/100 ml ±S D	RBC × 10⁶ ±S D	HCT % ±S D	MCV μ³ ±S D	MCHC ±S D	RETIC ±S D
Days							
1	19	19 3±2 2	5 14±0 7	61±7 4	119± 9 4	31 6±1 9	3 2±1 4
2	19	19 0±1 9	5 15±0 8	60±6 4	115± 7 0	31 6±1 4	3 2±1 3
3	19	18 8±2 0	5 11±0 7	62±9 3	116± 5 3	31 1±2 8	2 8±1 7
4	10	18 6±2 1	5 00±0 6	57±8 1	114± 7 5	32 6±1 5	1 8±1 1
5	12	17 6±1 1	4 97±0 4	57±7 3	114± 8 9	30 9±2 2	1 2±0 2
6	15	17 4±2 2	5 00±0 7	54±7 2	113±10 0	32 2±1 6	0 6±0 2
7	12	17 9±2 5	4 86±0 6	56±9 4	118±11 2	32 0±1 6	0 5±0 4
Weeks							
1–2	32	17 3±2 3	4 80±0 8	54±8 3	112±19 0	32 1±2 9	0 5±0 3
2–3	11	15 6±2 6	4 20±0 6	46±7 3	111± 8 2	33 9±1 9	0 8±0 6
3–4	17	14 2±2 1	4 00±0 6	43±5 7	105± 7 5	33 5±1 6	0 6±0 3
4–5	15	12 7±1 6	3 60±0 4	36±4 8	101± 8 1	34 9±1 6	0 9±0 8
5–6	10	11 9±1 5	3 55±0 2	36±6 2	102±10 2	34 1±2 9	1 0±0 7
6–7	10	12 0±1 5	3 40±0 4	36±4 8	105±12 0	33 8±2 3	1 2±0 7
7–8	17	11 1±1 1	3 40±0 4	33±3 7	100±13 0	33 7±2 6	1 5±0 7
8–9	13	10 7±0 9	3 40±0 5	31±2 5	93±12 0	34 1±2 2	1 8±1 0
9–10	12	11 2±0 9	3 60±0 3	32+2 7	91± 9 3	34 3±2 9	1 2±0 6
10–11	11	11 4±0 9	3 70±0 4	34±2 1	91± 7 7	33 2±2 4	1 2±0 7
11–12	13	11 3±0 9	3 70±0 3	33±3 3	88± 7 9	34 8±2 2	0 7±0 3

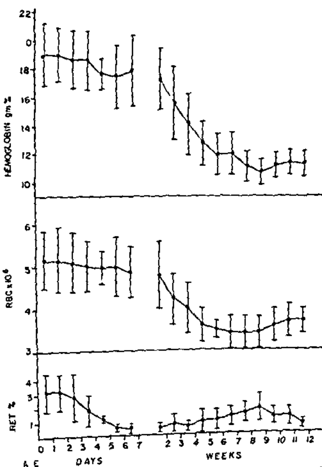


Fig 1 Hemoglobin concentration red cell count and reticulocyte count in the first 12 postnatal weeks Vertical bars represent standard deviation

RESULTS

Changes in the red cell count, hemoglobin concentration hematocrit MCV, MCHC and the reticulocyte count at daily intervals from day 1 to 7 and at weekly intervals from the second to the twelfth week are summarized in Table 1 and Fig 1

The mean red cell count on the first day of life was $5\ 14 \times 10^6$ It dropped steadily, reaching a low point of $3\ 4 \times 10^6$ in the 7th week remained unchanged for the following 3 weeks and showed a tendency to rise from the tenth week to the end of the period of observation

In Fig 2 results are expressed as the total number of circulating red cells derived from the red cell count and calculated blood volume It can be seen that the 7th week represents the turning point from a negative to a positive balance between red cell production and destruction

The hemoglobin concentration on day 1 was 19 3 g/100 ml It decreased steadily reaching a low point of 10 7 g/100 ml during the 9th week and subsequently rising to 11 3 g/100

ml during the remaining 3 weeks of observation

The MCHC on day 1 31 6 ′ was somewhat low by adult standards (Table 1) Following a gradual increase during the first 5–6 weeks it fluctuated around 34 for the remaining period of observation The difference between the MCHC value for day 1 or day 7 and any of the values for weeks 5 to 10 was significant ($p < 0.01$) while there was no significant week to-week variation during the period 5–12 weeks

The mean reticulocyte count on day 1 was 3 2 ₀ decreasing gradually from day 3 and reaching the 1% level on day 6 From the second to the 5th week it fluctuated between 0 5 and 1 0° A gradual rise was noted beginning in the 5th week and reaching a high of 1 8° in the 9th week. During the remaining 3 weeks of observation the reticulocyte count gradually returned to around 1 (Table 1 Fig 1)

The cell size distribution (Price Jones) curve on day 1 and day 28 is presented in Fig 3 The curves represent mean values for cell sizing obtained on 11 and 15 individual infants respectively A mean cell size distribution curve obtained in adults is presented for comparison Both adult and newborn distribution curves are skewed to the right a phenomenon commonly observed with electronic cell sizing (2 16) It can be seen that the distribution curve on day 1 is markedly shifted to the right As compared with the adult in whom 85 μ^3 represents the peak frequency in the 1 day-old infant this frequency is 102 5 μ^3 There is a considerable increase in the frequency distribution of macrocytes with a corresponding decrease in microcytes By 4 weeks the red cell size distribution curve approaches that of the adult

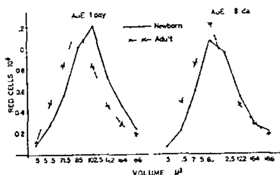


Fig 3 Mean cell size distribution (Price Jones) curves at age 1 day and 28 days Cell size distribution in adults is shown for comparison

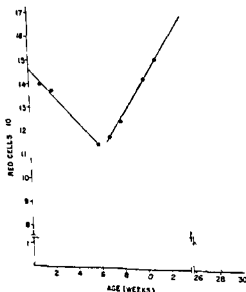


Fig ? Total number of circulating red cells in the first 1_ postnatal weeks

To follow the red cell size distribution curve as a *function* of age (Fig 4) the red cell population was somewhat arbitrarily divided into 3 groups

Cells having a volume of 71 5–102 5 μ^3 were grouped together as normocytes while smaller (34 0–57 5 μ^3) and larger (122 0–164 0 μ^3) cells comprised the microcytic and macrocytic

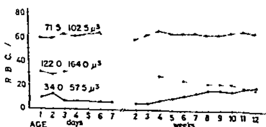


Fig 4 Changes in the percentage distribution of macrocytes normocytes and microcytes in the first 12 postnatal weeks

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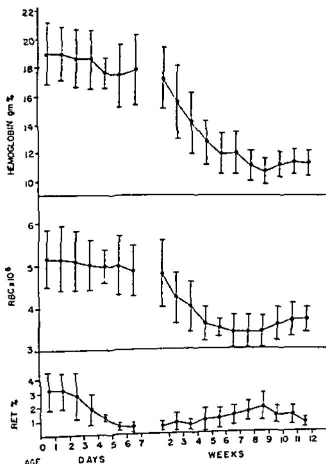


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ent at birth are followed within the first few postnatal days by a markedly reduced erythropoietic activity in the marrow and a low reticulocyte count (5 14) Our results conform with this general picture In some important details however our findings differ from those of earlier workers

The time of clamping of the umbilical cord is a variable which may affect red cell values in the postnatal period (4 8) Since early clamping is practiced in this hospital our infant population can be regarded as uniform in this respect The source of the blood samples is also of importance since red cell values are known to be higher in capillary than in venous blood Therefore the red cell values obtained by us represent normal values for capillary blood in an infant population with early clamping of the umbilical cord Indices calculated from these values such as MCHC are not affected by the source of blood. The dynamics of the red cell count or hemoglobin concentration may however vary somewhat with the source of blood especially during the first week of life

The trimenon anemia is a widely held concept The postnatal drop in the red cell count and hemoglobin concentration is generally believed to continue well into the third month (6 9 19) It is however clear from our data that while this is true of the hemoglobin concentration the low point in the red cell count is reached during the 7th week The lag of the hemoglobin concentration behind the red cell count is accounted for by the continuing decrease in MCV

That the 7th week represents a turning point is evident from a plot of the number of total circulating red cells (Fig 2) Although blood volumes were not actually determined by us calculations based on the red cell count weight and values for blood volume obtained by other workers for the age groups in question represent a close approximation

The data could be best fitted into two separate linear functions the first of which covered the period from birth to the seventh week Assuming complete cessation of red cell production after birth this plot would represent a red cell survival curve The absurdity of such an assumption is evident from an extrapolation of this plot which intercepts the abscissa at about 220 days (Fig 2) This figure is twice the normal life span of adult red cells and probably 3 times that of neonatal cells (7 11) It is therefore evident that red cells are produced in considerable numbers during the postnatal period For the first 6 weeks however production falls short of compensating for factors tending to lower the red cell count such as the short life span of the neonatal red cell and the expanding blood volume Since the rate of weight gain and the corresponding increase in blood volume are not markedly changed after 6 weeks the positive balance between red cell destruction and production achieved at this age can be attributed to an increase in the rate of production An increase in the mean red cell life span may also be a contributing factor

The reticulocyte count can be cited as further evidence for the persistence of considerable erythropoietic activity following the immediate postnatal slowdown It should be noted that after the initial drop at the end of the first week the reticulocyte count remained in the 0 5–1 0% range This corresponds to the normal adult level of reticulocytosis and is far from being indicative of a depressed erythropoiesis The rise in the reticulocyte count which is noticeable from the 5th week on indicates an increase in erythropoietic activity The rise is slow reaching its peak value at 9 weeks This is in keeping with a gradual increase in the level of red cell production rather than an abrupt "turning on of erythropoiesis in the third month

It has been shown that the red cell density distribution reflects cell age distribution (3 12) As in the adult the red cell density distribution curve in the newborn is sigmoid in shape However a comparison of the neonatal curve to that of adults should be approached with caution Since neonatal red

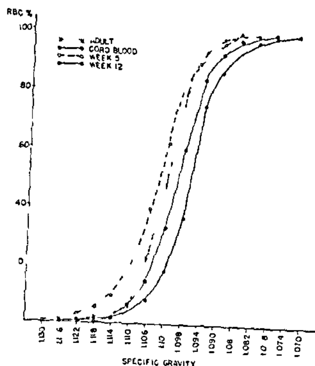


Fig 5 Mean red cell density distribution curves at birth 5 weeks and 12 weeks A density distribution curve of adult red cells is presented for comparison

groups respectively It can be seen that the percentage of macrocytes remained constant over the first 6 days On day 7 and during the second week there was actually some increase in the percentage distribution of macrocytes, followed by a steady decrease to the end of the period of observation The microcyte population was small initially From the 3rd week on it showed a steady increase, reciprocal to the decrease in macrocytes The normocyte population remained constant throughout most of the period of observation

Cumulative density distribution curves obtained at birth, 5 weeks and 12 weeks are presented in Fig 5 Typical sigmoid curves similar to those observed in adults are obtainable in the newborn However, minor fractions of cells with specific gravity outside the normal adult span are found in the newborn A fraction lighter than 0 090 is present in cord blood while a cell population heavier than 1 114 is found at 5 weeks

The density distribution curve at birth lies well to the right (light side) of the adult curve From birth to 5 weeks the curve shifts gradually to the left (heavy side), while from 5 to 12 weeks the trend is reversed and a shift back to the right is observed At 12 weeks the density distribution curve is again situated to the right of the adult curve

To determine the relationship between cell size and cell density distribution 13 full term infants aged 1–28 days, 10 premature infants of 30–34 weeks gestation and 4 adults were studied Cell sizing was done on the light and heavy fractions and revealed a preponderance of macrocytes (cells having a volume of 102 5–186 0 μ^3) in the light fraction of neonatal red cells This tendency was more marked in red cell populations obtained from premature infants, while in adults the cell size distribution in whole blood, light or heavy fraction did not differ significantly (Tables 2 and 3)

DISCUSSION

The state of erythropoiesis at birth and the changes it undergoes during the postnatal period are well known in their general outline At birth there is polycythemia and macrocytosis During the postnatal period there is a gradual decrease in the red cell count hemoglobin concentration and MCV (6) Marrow erythroid hyperplasia and reticulocytosis, pres

Table 2 *Distribution of macrocytes in various fractions of neonatal red cell*

Macrocytes ±S D	Fraction: Whole blood	Light	Heavy
Full term	75 7 ± 7 97	86 7 ± 11 8	63 2 ± 13 5
Premature	71 4 ± 8 05	83 2 ± 13 05	58 2 ± 8 84
Adult	37 2	37 4	37 0

Table 3 *MCV in various fractions of neonatal red cells*

MCV μ^3	Whole blood	Light	Heavy
Full term	112 2	129 5	103 9
Premature	120 7	146 3	111 4

percentage distribution decreased after the second week with a corresponding increase in microcytes.

The red cell density distribution curve in the neonatal period is sigmoid in shape The range of densities is however wider than in the adult The curve shifts gradually to the heavy side from birth to 5 weeks while from 5 to 12 weeks a shift back to the light side is observed Macrocytes preponderate in the light fractions

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cells differ from adult cells in their life span, the age specific gravity relationship characteristic of adult cells does not apply to the newborn Therefore, a given specific gravity does not necessarily represent the same red cell age in the adult and the newborn This point is emphasized by the finding of small populations of neonatal cells having specific gravities outside the normal adult span at both extreme ends of the density distribution curve (Fig 5)

These objections do not apply when density distributions are compared within the newborn period The shift to the heavy side from birth to 5 weeks demonstrates clearly a deficit in young cells and a preponderance of old cells during this interval This is compatible with a situation in which cell death exceeds the production of new cells The density distribution at 12 weeks, on the other hand, is characteristic of the rapidly growing infant, continuously expanding its blood volume Red cell production exceeds destruction and younger cells predominate

The nature and fate of the macrocyte population present at birth is of interest The macrocytes may represent a primitive fetal type of red cell, differing in size as well as in other properties from cells produced later during an adult phase of erythropoiesis The very large volume of red cells in the early stages of gestation and its diminution as gestation progresses (15) support this hypothesis We have found (unpublished data) the population of macrocytes, especially of the very large cells, to be considerably larger in premature infants of 28–32 weeks gestation than in term infants

Macrocytosis at birth, on the other hand may reflect the presence of a large population of young red cells, resulting from the intensive erythropoiesis characteristic of the prenatal period The young red cell is known to be larger and to decrease in volume with increasing age (18) Furthermore, Brecher & Stohlman (2) found that, following severe erythroid stimulation, red cells are produced which are macrocytic and that these stress macrocytes have a shortened life span Our finding of a predominance of macrocytes in the lighter, i e younger, fractions is consistent with this latter concept It should be noted however, that early fetal red cells may represent a qualitatively different population and have densities lower than adult cells of corresponding age

Some increase in the percentage of macrocytes was observed during the first week of life A similar increase was found by Wunderlich & Hinkel (19) and may be explained by the maturation and release into the peripheral blood of erythroid elements present in the marrow at birth From the second week on the number of macrocytes decreases gradually and a simultaneous increase in microcytes takes place Whether this change in the cell size distribution reflects elimination of short lived stress macrocytes and their replacement by a cell population of smaller size or a decrease in size associated with cell aging cannot be answered with certainty from our data The gradual postnatal increase in MCHC is consistent with cell shrinking due to aging of the predominantly macrocytic cell population present at birth and would therefore favor the latter alternative

SUMMARY

The red blood picture was studied in detail from birth to 12 weeks The low point in the red cell count was reached in the seventh week which represented the turning point from a negative to a positive balance between cell production and destruction From the dynamics of the total number of circulating red cells it was concluded that although erythropoiesis slows down after birth a considerable amount of red cell production takes place during the first few postnatal weeks

The MCHC increased significantly over the first 5–6 postnatal weeks and remained constant thereafter

Macrocytes predominated at birth Their

percentage distribution decreased after the second week with a corresponding increase in microcytes

The red cell density distribution curve in the neonatal period is sigmoid in shape The range of densities is however wider than in the adult The curve shifts gradually to the heavy side from birth to 5 weeks while from 5 to 12 weeks a shift back to the light side is observed Macrocytes preponderate in the light fractions

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CYSTATHIONINURIA IN A WELL BABY POPULATION

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Cystathionine is a sulphur-containing amino-acid, classed as non-essential since it is formed from methionine It normally occurs in tissue proteins, is found in trace amounts in blood and urine, and is not significantly resorbed by the renal tubule A defect in the enzyme cystathioninase which cleaves cystathionine has been correlated with the appearance of large amounts of cystathionine in the blood and in the urine (10) The disorder is apparently inherited as a Mendelian recessive character The reports of 14 patients suffering from this disorder have been summarized (14) Cystathionine has also been found in smaller amounts in the urine of some of the relatives of these individuals These cystathionine excretors are presumptively heterozygous for the enzymic defect

Cystathioninuria has been described in association with pyridoxine deficiency states (15) with gross liver failure (11) and with neuroblastoma (9)

More recently cystathioninuria homocystinuria and hypomethioninaemia have been demonstrated in an infant with a defect in the activity of the homocysteine remethylating enzyme (12) This is probably due to an abnormality in vitamin B_{12} metabolism

Von Studnitz (20) found cystathioninuria in 6 out of 230 infants hospitalised for investigation of suspected metabolic disorders, convulsions or mental retardation

There has been to date no survey of a significant number of normal infants to determine the incidence of excess urinary cystathionine in an otherwise unselected population

MATERIAL AND METHODS

In the course of the urinary metabolic screening survey carried out in New South Wales (19) the urine of 35 809 infants was examined over a 6 month period by means of one way paper chromatography and ninhydrin staining Suspected abnormal cystathionine excretion was further investigated by high voltage electrophoresis thin layer chromatography in two dimensions and by its reaction with iodoplatinate (16) Quantitative studies were made on a Beckman Unichrom amino acid analyser Fasting plasma amino acids were quantitated after removal of the proteins with picric acid (8)

RESULTS

Cystathioninuria was recognised in 15 infants of the 35 809 screened at approximately 6 weeks of age Analysis of follow up urine specimens obtained when the fifteen infants were approximately 5 months old revealed that eleven infants (5 males and 6 females) showed no demonstrable cystathioninuria, 2 male infants showed traces of cystathionine in the urine while a further 2 males (D M and R S) showed a persistent and heavy cystathioninuria

Enquiries of the parents of these 15 children and of their physicians failed to elicit a history

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Table 1 *Amino acid analysis of liquid urine samples in case 1*

Age	Treatment	Cystathionine (mg) per g creatinine	Homocystine (mg) per g creatinine
20 weeks	None	1 943	150
22 weeks	100 mg pyridoxine daily for 2 weeks	55	Nil
9 months	Pyridoxine suspended for 4 weeks prior to test	1 043	Nil
10 months	Methionine 100 mg per kg body weight (single dose)	1 309	12
12 months	500 mg pyridoxine daily for 4 weeks	21	Nil

of abnormality in these infants Three infants were examined by one of us (P G P) and no clinical abnormalities were revealed Further studies on the 2 infants with persistent cystathioninuria were undertaken

CASE REPORTS

Case 1 (D M S315085 male) was born of unrelated parents and weighed 3 400 g at full term No neonatal problems were encountered and he was artificially fed from birth At 7 weeks of age a urine sample revealed excessive cystathionine excretion which was confirmed by a repeat test at 16 weeks The child was alert and responsive but its motor milestones were slightly delayed namely sitting alone at 8 months and beginning to crawl at 12 months

A positive cyanide nitroprusside test (4) was obtained on a liquid urine sample obtained at 20 weeks Quantitative amino acid analysis revealed elevated levels of both cystathionine and homocystine in the urine while cystine excretion was normal (Table 1) Cystathionine (0 050 µmoles/ml) was detected in fasting plasma at 20 weeks

Pyridoxine hydrochloride (250 mg daily) was given orally and a urine test 2 weeks later showed a marked reduction in cystathionine and no demonstrable homocystine in the urine At 8 months of age pyridoxine was suspended for 4 weeks and the concentration of cystathionine in the urine again rose (Table 1) Homocystine was not demonstrated in the urine Haematological studies revealed no abnormality The vanillylmandelic acid level in the urine was normal and no methylmalonic acid was detected

Cystathionine was not detected in early morning urine specimens of the parents or of the 3 siblings

After 500 mg of pyridoxine daily for 4 weeks the cystathionine excretion at the age of 12 months was reduced to almost normal levels

Loading tests When daily administration of pyridoxine to the propositus had been suspended for 4 weeks he and his parents were given methionine (100 mg/kg body weight) with the evening meal and early morning urine specimens were collected the next day Cystathionine was detected in trace quantity in the urine samples from the parents In the infant, then 10 months of age cystathionine excretion rose to over 1 g/g creatinine and homocystine was again demonstrable The cyanide nitroprusside test was weakly positive

Case 2 (R S S315875 male) the firstborn of healthy and unrelated parents weighed 3 020 g at full term The baby was artificially fed thrived normally and achieved his developmental milestones at normal times Excessive cystathionine excretion was first demonstrated at 5 weeks of age and was confirmed at 19 weeks The amino acids were quantitated in a liquid urine sample at 21 weeks (Table 2) and cystathioninuria was confirmed glycine excretion was elevated but homocystine was not demonstrated

Pyridoxine hydrochloride (250 mg daily) administered orally was given by mouth for 2 weeks and the cystathionine excretion was reduced but the excretion of glycine increased This elevation of glycine excretion was accompanied by excesses in proline and hydroxypro-

line excretions Four weeks after the cessation of pyridoxine treatment the cystathionine excretion returned to pretreatment levels whereas glycine excretion returned to normal values

Fasting plasma amino acids quantitated at 21 weeks revealed an elevated cystathionine level (0 027 μmoles/ml), normal glycine values, and absence of homocystine was demonstrated

Urine samples from the parents showed an excretion of cystathionine in the father of 60 mg/g of creatinine on one occasion, but no demonstrable cystathioninuria in the mother

Loading tests (as in case 1) revealed a trace of cystathionine in the mother's urine but none was demonstrated in the father s urine on this occasion

Table 2 *Amino acid analysis of liquid urine samples in case 2*

Age	Treatment	Cystathionine (mg) per g creatinine	Glycine (mg) per g creatinine
21 weeks	None	1 909	358
23 weeks	100 mg Pyridoxine daily for 2 weeks	37 7	585
27 weeks	Pyridoxine suspended for 4 weeks prior to test	836	84
Normal		12 5	-00

DISCUSSION

The incidence of cystathioninuria in a population of healthy infants has not been determined previously The detection of excessive cystathionine excretion by the present method clearly underestimates its incidence The lack of specificity obtained by ninhydrin staining of the chromatograms makes a considerable elevation of cystathionine necessary before an abnormality is suspected It would appear likely, however that infants with considerable cystathioninuria would be detected

Genetic studies (7, 21), which have been conducted on the families of patients who are excreting large amounts of cystathionine and whose plasma showed elevated levels of cystathionine, suggest that these individuals are homozygous for an enzyme defect The heterozygotes may show a slight excess of urinary cystathionine and this may become more apparent after a loading dose of methionine, the precursor of cystathionine (13)

The defect in the enzyme cystathionase in the liver of the presumptive homozygous individuals has been shown to respond *in vitro* to an excess of pyridoxine (6)

The two infants ascertained who showed both a heavy cystathioninuria and cystathioninaemia responding to pyridoxine administration, would appear to be homozygous for this enzyme defect The demonstration of cystathioninuria in each of the four parents, at one time or other, indicates that they may be heterozygous for this enzyme defect

The clinical significance of homozygous cystathioninuria has been rendered obscure by the methods employed previously in ascertainment of cases Of the described cases only 2 patients were subjected to fortuitous urinary amino acid chromatography (13) The combination of these patients, the normal relatives of ascertained patients (5, 14), and the present patients suggests that if this condition has some clinical significance then it has to date, escaped recognition The suggestion that cystathioninuria is a benign disorder (6 13) is supported by the present study

The study of Scriver & Hutchison (15), confirmed in man that vitamin B_6 deficiency will produce cystathioninuria No evidence of such deficiency was obtained in any of the infants studied in the present series Cystathioninuria has also been reported in patients with galactosaemia hepatoblastoma and neuroblastoma There has been no evidence to suggest these possibilities in any of the infants in the present series

The infants in whom transient cystathioninuria has been demonstrated may be heterozygotes for this condition or they may have had a transient vitamin B deficiency These possi

bilities remain unexplored Similarly the two infants in whom trace amounts of cystathionine persisted in the urine may be heterozygotes It is less likely however, that they are vitamin B_6 deficient as they were receiving adequate diets were in a good state of nutrition, and were not receiving any recognised pyridoxine antagonists

The presence of a positive cyanide nitroprusside test in a patient with cystathioninuria has previously been reported (3 12 18) One case was characterised biochemically by the presence of hypomethioninaemia and the excretion of methylmalonic acid in the urine (12) This disorder has been shown to be a different entity from those presently under consideration where neither of these conditions were present. In Berlow s patient no cause for the positive cyanide nitroprusside test could be found and in particular homocystine was not present in the urine (2) Both the cystathioninuria and the positive cyanide nitroprusside test responded favourably to the administration of pyridoxine in Berlow s case Tada et al (18) found a small amount of homocystine insufficient to cause a positive cyanide nitroprusside reaction in the urine of his cystathioninuric patient In this case the biochemical abnormalities did not respond to pyridoxine

The amount of homocystine in the urine of our case 1 (D M) was much higher than in Tada s patient and was of sufficient quantity to produce a positive cyanide nitroprusside test This finding was associated with very high levels of both plasma (0 050 μmol per ml) and urinary cystathionine (1 943 mg per g of creatinine) After a course of pyridoxine therapy the cystathionine in the urine fell markedly and the homocystine disappeared Although cystathionine excretion again rose after pyridoxine was suspended homocystine did not reappear in the urine until a methionine load test further increased the cystathionine excretion

Although it has been shown in experimental animals that cystathioninuria and homocystinuria can be caused by vitamin B_6 deficiency (1) the diet of D M has always been adequate It has been estimated that at the time when homocystinuria was present he was receiving 0 5 mg of vitamin B_6 daily from his milk formula alone in addition to that received from other dietary sources such as cereals Also no other clinical effects of vitamin B_6 deficiency such as convulsions or anaemia have been observed The finding of a normal urine taurine level of 22 mg per g creatinine argues against vitamin B_6 deficiency being the cause of this child s biochemical abnormalities (17)

SUMMARY

Screening of 35 809 infants by one way chromatography revealed 15 cases of excessive cystathionine excretion Evidence is presented to suggest that two of these are homozygous for primary cystathioninaemia Homocystinuria was present transiently in one of these two infants It is concluded that cystathioninuria is a benign disorder and appears to be much more common than previously recognised

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THE THIRD FONTANELLE

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In a continuing study of Down s syndrome in the newborn infant the presence of the third fontanelle was noticed fairly frequently This high incidence was also noticed in infants pre senting with the congenital rubella syndrome It was therefore decided to make a study of this particular single defect in the newborn in fant in the variety of conditions that occur in this hospital. It has been shown that major ab- normalities are more frequent in infants pre senting with minor congenital defects (6)

The third fontanelle is a bony defect along the sagittal suture about 2 cm anterior to the posterior fontanelle (3 4 5) it is not a true fontanelle being related exclusively to the parie tal bones This fontanelle can often be seen on X rays as a depression in the contour on a lat eral view (Fig 1) and a diamond shaped defect on the antero-posterior view which however is often obscured by overlying bones It can be seen at autopsy in some infants

MATERIAL AND METHOD

In the Kandang Kerbau Hospital Singapore where this study was made over 31 000 infants were de livered in 1969 This study was made over a period of 6 months Infants in the first week of life were se lected at random and examined clinically to detect any physical defect

The infants were then divided into the normal group with no physical defects which served as con trols and a second group consisting of infants in whom some abnormality was detected. A separate group of infants with Down s syndrome was also included in this study this group was collected over a longer period of time as compared with the con trols. During this period too a fairly large number of infants were noticed to be suffering from the ex panded rubella syndrome these infants were also included in this study

The presence of the third fontanelle was assessed by palpating the skull of the infant along the sagittal suture from the anterior fontanelle to the posterior fontanelle for the presence of a defect about 1 / to 2 / cm anterior to the posterior fontanelle The procedure was repeated carefully twice Only definite defects were accepted as evidence of the presence of the third fontanelle Notches and doubtful findings were excluded in this study The defect was classified as large if the coronal diameter was more than 1 cm and small if the diameter was 1 cm or less The results were subjected to statistical analysis for sig nificance

RESULTS

Altogether 1 930 controls were examined con- sisting of 1 518 Chinese 280 Malays 120 In- dians and 12 of mixed or other ethnic groups The incidence in the separate ethnic groups did not show significant differences all the in fants of different ethnic groups were therefore studied together The incidence according to weight was highest in the group weighing 2 001 to 2 500 g (Table 1) The incidence of the third fontanelle seemed to be unusually high in the group presenting with the congenital rubella syndrome (Table 2) compared with the total overall incidence of the controls ($p <$ 0 001) or the weight group of the controls with the maximum incidence ($p < 0\ 001$) This sig- nificantly high incidence was also present in the group with Down s syndrome ($p < 0\ 001$)

Of the 22 infants with the expanded rubella syndrome that were examined, 16 were less

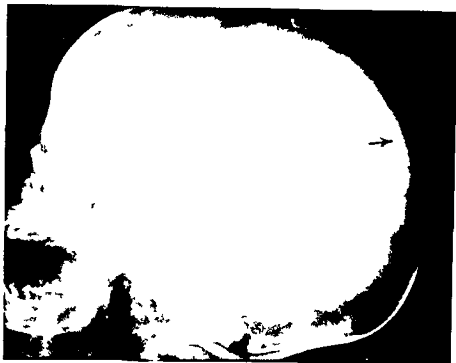


Fig 1 Third fontanelle indicated (*arrow*) in lateral view of the skull

than 2 000 g in weight at birth and 3 between 2 001 and 2 500 g only 3 being more than 2 500 g Twenty of these infants were over 38 weeks' gestation at delivery

The size of the third fontanelle was very variable the largest in the control group was 3 cm while in the congenital rubella group the largest third fontanelle (2 8 cm) was found in a 1 290 g small for date infant In the infants with Down s syndrome the largest fontanelle was 3 2 cm

The incidence of the third fontanelle in the group consisting of various abnormal conditions (Table 3) was higher than that in the controls but this association was not significant (0 1 > p > 0 05) With regard to the individual conditions in this group the numbers involved were too small to allow any definite conclusions to be drawn

DISCUSSION

The parietal bone ossifies in membrane from one or two centres in the region of the future parietal eminence at about the seventh or eighth week of intra uterine life (7) A network of fine bony trabeculae spreads from the region of the eminence in the form of radiat

Table 1 *Incidence in normal infants*

Weight group (g)	Total seen	No with the third fontanelle					
		Actual numbers			Incidence (per 1000)		
		Large	Small	Total	Large	Small	Total
1 501 and below	40	1	1	2	25	25	50
1 501-2 000	132	9	12	21	68	91	159
2 001-2 500	303	36	27	63	120	90	210
2 501-3 000	561	47	43	90	84	77	161
3 001-3 500	599	41	27	68	69	45	114
3 501-4 000	233	13	9	22	56	39	95
4 001 and above	62	4	0	4	66	0	66
Total	1 930	151	119	270	78	62	140

Table 2 *Incidence in abnormal infants*

	Total seen	No with the third fontanelle					
		Actual numbers			Incidence (per 1000)		
		Large	Small	Total	Large	Small	Total
Down s syndrome	84	35	14	49	416	168	584
Congenital rubella syndrome	22	14	5	19	636	227	863

ing bony spicules The spread towards the central plane is slow and the defect in this region constitutes the third fontanelle The third fontanelle normally disappears by the seventh month of fetal life (3) but it occasionally persists as the third fontanelle in infancy

The incidence of the third fontanelle has been found to be 44–64 per 1 000 (3 4 5) In the present study an overall incidence of 140 per 1 000 is very much higher than the above mentioned investigations This reason for this great disparity is not understood, however the present incidence is much lower than that arrived at by Adair & Scammon (1) These authors by tracing the sagittal suture on a piece of linen placed over it found a verv high incidence of 307 per 1 000 in a study of 241 infants in the first month of life more than twice the numb r of the present study The detection of the third fontanelle by the method of palpation is not difficult though cases of craniotabes may present some difficulties With careful light, and repeated palpation of the sagittal suture in such cases a correct diagnosis can be arrived at reliably besides the incidence of craniotabes is not high enough to influence the overall results

In another study (3) a higher incidence was found in prematures though this difference was not significant In the present study no such incidence was found The highest incidence was found in the group weighing from 2 001 g to 2 500 g The group weighing 1 500 g and le s in fact had the lowest incidence

In Down s syndrome th re was an unusually high incidence in the presence of the third fontanelle compared with the overall incidence of the controls ($p < 0\ 001$) or compared with the weight group with the highest incidence ($p < 0\ 001$) This finding is in agreement with that of Chemke & Robinson (3) but differs from that of Hoyle & Franklin (4) Carter & MacCarty (2) mentioned that it is often present in Down s syndrome while Penrose & Smith (8) have commented on its occasional presence in this syndrome The present study of 84 newborns with Down s syndrome shows quite definitely the unusual frequency of the phenomenon

The incidence of the third fontanelle seems to be also remarkably high in the congenital rubella syndrome this is believed to be the first occasion where this microsign has been shown to be unusually high in this condition though Chemke & Robinson (3) mentioned its presence in one infant with this condition Its frequency is so high that it can be regarded as

Table 3 *Incidence in other abnormal infants*

	No with 3rd fontanelle		
	No seen	Large	Small
Exomphalos	3	1	0
Ellis van Creveld syndrome	1	1	0
Pierre Robin syndrome	2	1	1
Congenital syphilis	1	0	0
Primary macroglossia	3	0	0
Arnold Chiari syndrome	1	0	0
Partial albinism	2	0	0
Preauricular tag	5	1	1
Skin aplasia scalp	4	1	0
Anophthalmia	1	0	0
Oesophageal atresia	1	0	0
Trisomy 17–18	1	0	0
Chordee	2	0	0
Neonatal teeth	4	1	0
Total	31	8	

an almost constant feature of the syndrome Twenty of the infants were full term though the majority were below 2 000 g in weight Perhaps this intra uterine growth retardation also interfered with the ossification of the parietal bone resulting in the presence of the third fontanelle No definite cases of other forms of intra uterine infection except one of congenital syphilis were available for examination during this period, however it has been shown (3) that the IgM levels were not raised in cases presenting with it This would indicate that the third fontanelle is associated with congenital rubella rather than with intra uterine infection in general

In the group of infants with various other abnormalities, the incidence of the third fontanelle was increased but this was not significant statistically As for the individual conditions, within this group, further studies on larger numbers are necessary before any definite conclusions can be drawn

SUMMARY

One thousand, nine hundred and thirty apparently normal newborn infants were examined for the presence of the third fontanelle In addition other newborn infants with abnormalities were also screened for the same defect

The incidence of the third fontanelle was high, in this study, among the normals, compared with past studies The incidence seemed highest in the group weighing 2 001 to 2 500 g In the abnormal group, the incidence was very significantly raised in infants suffering from the congenital rubella syndrome or Down s syndrome, it was raised but not significantly in infants presenting with various other abnormalities

The presence of the third fontanelle should alert the examiner to the possibility of other defects in the infant

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THE HEAD CIRCUMFERENCE IN INFANTS AND OTHER MEASUREMENTS TO WHICH IT MAY BE RELATED

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The routine examination of a baby in a private house welfare clinic or hospital should include the measurement of the maximum head circumference for the head size reflects the growth of the cranial contents If the growth of the brain is defective the head is likely to be small and if there is hydrocephalus megalencephaly or hydranencephaly the head is likely to be large Smallness of the head may be a valuable pointer to mental subnormality and a large head to hydrocephalus and so to the appropriate treatment

A small or large head could be merely a familial feature or a reflection of the size of the child for one would expect a large child to have a larger head than a small child and a small child to have a smaller head than a large child Hence one must always relate the size of the head to the size of the baby Two of us (2) showed that there is a strong relationship between the head circumference and the weight In this paper we have set out to determine whether the head circumference is better related to the weight chest circumference supine length or crown rump length

MATERIAL AND METHODS

Anthropometric data concerning 50 boys and 56 girls living in Sheffield were collected by one trained nurse for the purpose of the study Measurements were made at birth 6 weeks, 3 months and 6 months.

The maximum head circumference was measured by a cloth tape measure which was repeatedly checked for accuracy

The chest circumference was measured by the tape around the chest at the level of the xiphi sternal joint. The supine length and crown rump length were measured on a supine length table The nude weight was recorded

Tables 1 and 2 show the mean and standard deviation of the head circumference weight, chest circumference supine length and crown rump length Eight boys and two girls had a low birth weight (<2500 g) and this accounts for the lower mean birth weight in boys than girls

The correlation coefficient and the regression equation (with its standard error) were determined between head circumference and each parameter at the four different ages in each sex separately (Tables 3 and 4) The 6 week follow up included all children aged 6 weeks ± 2 weeks the 3 months and 6 months follow ups included all children aged 3 months and 6 months ± 3 weeks

RESULTS

From Tables 3 and 4 we observe that the correlation and the regression coefficients of the head circumference on each parameter are highest at birth (especially in boys) and at 6 weeks and are then less

The correlation of head circumference and gestational age was low at birth in both sexes probably because of the difficulty in assessing the gestational age (for boys $r = 0.416$ for girls $r = 0.447$)

The head circumference showed the highest correlation with the weight in girls at all ages studied while in boys it showed the highest correlation with the weight at birth and at 3 months with the chest circumference at 6 weeks and 6 months

Table 1 *Means and standard deviations of different parameters at the different follow ups in boys*

	Head circumference (cm) mean ± S D	Weight (kg) mean ± S D	Chest circumference (cm) mean ± S D	Supine length (cm) mean ± S D	Crown rump length (cm) mean ± S D
Birth ($n=50$)	34 4 ± 1 5	3 172 ± 0 572	30 8 ± 2 0	48 7 ± 2 1	33 1 ± 2 0
Six weeks ($n=37$)	38 1 ± 1 7	4 622 ± 0 677	36 3 ± 2 8	52 9 ± 2 1	36 1 ± 1 9
Three months ($n=43$)	41 1 ± 1 8	6 497 ± 0 932	41 3 ± 2 7	58 0 ± 2 8	39 9 ± 2 1
Six months ($n=50$)	44 3 ± 1 9	8 550 ± 1 147	45 2 ± 2 9	64 9 ± 2 4	43 6 ± 1 7

Table 2 *Means and standard deviations of different parameters at the different follow ups in girls*

	Head circumference (cm) mean ± S D	Weight (kg) mean ± S D	Chest circumference (cm) mean ± S D	Supine length (cm) mean ± S D	Crown rump length (cm) mean ± S D
Birth ($n=56$)	34 2 ± 1 3	3 211 ± 0 486	31 0 ± 1 7	48 1 ± 2 1	33 1 ± 1 6
Six weeks ($n=41$)	37 8 ± 1 2	4 372 ± 0 560	35 8 ± 2 0	52 4 ± 1 6	35 8 ± 1 6
Three months ($n=52$)	39 9 ± 1 2	5 727 ± 0 745	39 5 ± 2 0	56 4 ± 2 4	38 8 ± 2 2
Six months ($n=56$)	42 8 ± 1 3	7 609 ± 0 973	43 5 ± 2 3	62 8 ± 2 3	42 1 ± 1 7

Table 3 *Correlation coefficient and the regression equation (with its standard error) of head circumference or other parameters in boys*

r = correlation coefficient Reg eq = regression equation S E = standard error

		Head circumference and weight	Head circumference and chest circumference	Head circumference and supine length	Head circumference and crown rump length
Birth	r	0 818	0 738	0 792	0 789
	Reg eq	$y = 27\ 693 \quad 2\ 104x$	$y = 17\ 282 + 0\ 555x$	$y = 7\ 375 + 0\ 555x$	$y = 15\ 580 + 0\ 568x$
	S E	0 847	0 993	0 899	0 904
Six weeks	r	0 775	0 824	0 734	0 746
	Reg eq	$y = 28\ 997 + 1\ 978x$	$y = 19\ 873 + 0\ 503x$	$y = 6\ 664 + 0\ 595x$	$y = 13\ 570 + 0\ 680x$
	S E	1 093	0 978	1 174	1 151
Three months	r	0 670	0 585	0 474	0 563
	Reg eq	$y = 32\ 980 + 1\ 255x$	$y = 25\ 200 + 0\ 386x$	$y = 23\ 742 + 0\ 300x$	$y = 22\ 694 + 0\ 462x$
	S E	1 297	1 417	1 538	1 444
Six months	r	0 532	0 513	0 461	0 574
	Reg eq	$y = 36\ 908 + 0\ 867x$	$y = 29\ 373 + 0\ 330x$	$y = 20\ 735 + 0\ 364x$	$y = 17\ 121 + 0\ 624x$
	S E	1 583	1 604	1 659	1 530

Table 4 *Correlation coefficient and the regression equation (with its standard error) of head circumference on other parameters in girls*

r – correlation coefficient Reg eq – regression equation S E. – standard error

		Head circumference and weight	Head circumference and chest circumference	Head circumference and supine length	Head circumference and crown rump length
Birth	r	0 705	0 643	0 554	0 571
	Reg eq	$y = 28\ 178 + 1\ 870x$	$y = 19\ 008 + 0\ 489x$	$y = 17\ 913 + 0\ 338x$	$y = 19\ 154 + 0\ 454x$
	S E	0 913	0 987	1 072	1 057
Six weeks	r	0 789	0 716	0 618	0 581
	Reg eq	$y = 30\ 100 + 1\ 750x$	$y = 21\ 794 + 0\ 446x$	$y = 13\ 237 + 0\ 468x$	$y = 21\ 294 \pm 0\ 460x$
	S E	0 76[illegible]	0 866	0 975	1 009
Three months	r	0 664	0 584	0 636	0 534
	Reg. eq	$y = 33\ 94$[illegible] $+ 1\ 047x$	$y = 26\ 225 + 0\ 347x$	$y = 21\ 369 + 0\ 330x$	$y = 28\ 901 + 0$ [illegible]$84x$
	S E	0 879	0 955	0 908	0 994
Six months	r	0 600	0 535	0 493	0 443
	Reg eq	$y = 36\ 455 + 0\ 8$[illegible]$8x$	$y = 28\ 956 + 0\ 317x$	$y = $[illegible]$4\ 440 + 0\ 292x$	$y = 28\ 404 + 0.341x$
	S E	1 073	1 133	1 167	1 202

In girls the head circumference correlated next best with the chest circumference at birth 6 weeks and 6 months and in boys at 3 months The correlation of head circumference and supine length or crown rump length was not so high

Since the weight and chest circumference showed the highest correlation with the head

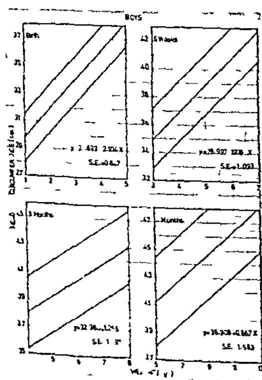


Fig. 1 Regression lines ± standard errors of head circumference on weight in boys.

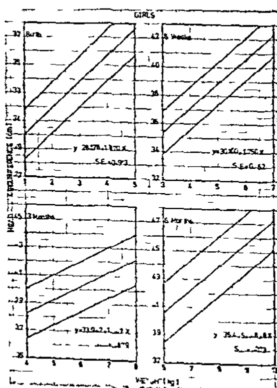


Fig 2 Regression lines ± standard errors of head circumference on weight in girls.

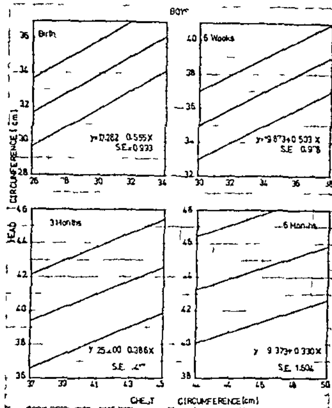


Fig 3 Regression lines ±2 standard errors of head *circumference on chest circumference in boys*

circumference at most of the ages studied and because they are easily measured graphs were drawn (Figs 1, 2 3 and 4) showing the linear regression line ±2 standard errors of head circumference on weight or chest circumference at each age and in each sex separately If the actual head circumference is plotted on the graph appropriate for age and sex and if it lies within the limits of ±2 standard errors (within 95% confidence limits), it is considered to be within normal limits in conjunction with other relevant clinical features The expected head circumference can be *determined directly from the graphs* (Figs 1, 2 3 and 4) or by substituting weight or chest circumference for *x* in the regression equations (Tables 3 and 4)

DISCUSSION

The head circumference correlates highly with the body size in the first 6 months of life It is useful to use only one graph, appropriate for the age and sex, relating head circumference to weight or chest circumference, instead of using two graphs relating each parameter to chronological age Illingworth & Lutz (2) stated that the relationship between head circumference and weight is uncertain in cases of failure to thrive and malnutrition the head tending to be larger than usual in relation to the weight Dean (1) showed that the head circumference and chest circumference are the least affected by malnutrition In the present study, the chest circumference shows a high correlation with head circumference, nearly as high as that of the weight

In conclusion it would seem that for routine purposes the head circumference should be related to the baby s weight and if desired to the chest circumference

SUMMARY

Head circumference was related to weight, chest circumference supine length and crown rump length at birth, 6 weeks 3 months and 6 months in fifty boys and fifty six girls

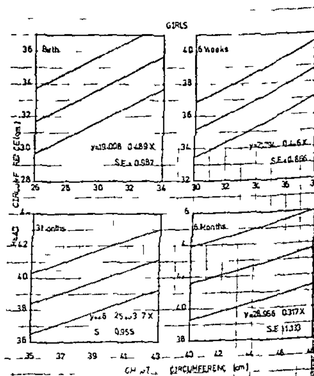


Fig 4 Regression lines ±2 standard errors of head circumference on chest circumference in girls

1 The head circumference is highly correlated with body size especially at birth and 6 weeks

2 The head circumference shows the best correlation with weight and chest circumference particularly the weight in both sexes

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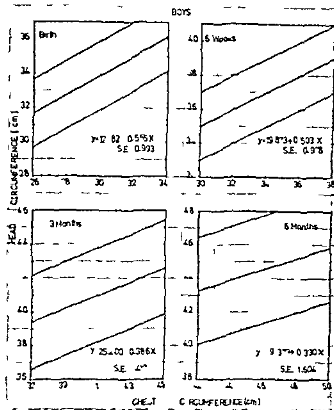


Fig 3 Regression lines ±2 standard errors of head circumference on chest circumference in boys

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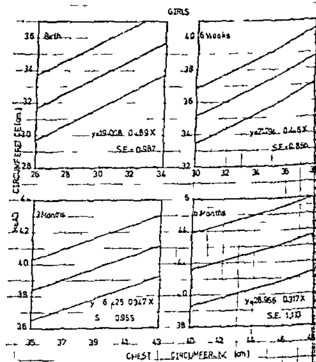


Fig 4 Regression lines ±2 standard errors of head circumference on chest circumference in girls

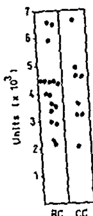


Fig 1 Duodenal juice alkaline phosphatase (*DAP*) activity in rachitic children (*RC*) and control children (*CC*)

from the different sources can be separated by electrophoresis (4 6) Intestinal alkaline phosphatase is found in the serum of only one fifth of the normal adult population and when present it contributes 15 to 30% of the total activity (9) This most likely originates from the intestinal mucosa (5)

Although some decrease of this enzyme activity in the jejunum and kidney was suggested by Cheesman et al in their experimental work on vitamin D deficient rats no changes could be found in the duodenum (2) By assaying the different tissues for alkaline phosphatase activity in rachitic rats we have confirmed their findings with the exception of jejunal alteration (8) Neither was any difference demonstrated between the DAP activities of rachitic children and those of the control subjects Duodenal juice alkaline phosphatase activity probably derives from the intestinal mucosa and hepatobiliary tree Since the alkaline phosphatase activity of the duodenal mucosa and the liver is not altered in the vitamin D deficient rats (2 8) our findings in children is not unexpected

Since it is known that serum alkaline phosphatase activity may not be increased in malnourished children with rickets (10) the DAP activity of five malnourished rachitic children was compared with our control values and no statistically significant difference could be found ($p > 0.05$) When the serum alkaline phosphatase values of our rachitic cases were compared with the corresponding DAP activities no correlation was found either As mentioned above, we have shown that leukocyte alkaline phosphatase activity in children with rickets due to vitamin D deficiency is not significantly different from the control values (7)

From these results it might be suggested that the characteristic serum alkaline phosphatase rise in rickets is not a reflection of changes of this enzyme in all tissues where alkaline phosphatases are present Another discrepancy between this enzyme level in serum and tissue has been reported in hypophosphatasia where serum level is markedly diminished in spite of normal activity (3 11) in duodenal juice and mucosa However the isoenzyme technique will probably be more valuable for a study of these problems

SUMMARY

Duodenal juice alkaline phosphatase activity of 17 children with rickets was assayed and not found to be different from the control values Malnutrition did not seem to affect this activity

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ALKALINE PHOSPHATASE ACTIVITY OF DUODENAL JUICE IN RICKETS DUE TO VITAMIN D DEFICIENCY

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Alkaline phosphatase is one of the first enzymes to be explored in human disease and its clinical significance is fairly well known The rise of serum alkaline phosphatase along with the decrease of serum phosphorus level, is a characteristic finding in rickets but tissue phosphatase activity in this disease has not been investigated (4) It is surprising to find that although this enzyme activity in the tissue is fairly well documented in hypophosphatasia (1 3, 11) which is comparatively rare and more recently described condition, there is very little in the literature about this activity in rickets

In a previous publication we described studies on the leukocyte alkaline phosphatase level in children with rickets due to vitamin D deficiency (7) In the present study the duodenal juice alkaline phosphatase levels (DAP) were assayed in patients with rickets due to vitamin D deficiency and compared with those of normal, age matched controls

MATERIAL AND METHODS

Duodenal juice was obtained for control values from 10 children three to 24 months of age who were admitted to the hospital for minor surgical procedures They had no evidence of rickets on clinical radiological or chemical examination and they were free of apparent infections There were 17 patients with rickets 5 of whom were also malnourished Twelve of these had acute infections of varying types The ages of these patients ranged from 50 days to 15 months In all cases rickets was confirmed by X ray examination of the wrists and marked elevation of serum alkaline phosphatase was found in eight of them

The alkaline phosphatase activity of the juice was determined by the technique of Valentine & Beck (12), using a Coleman Junior spectrophotometer (at 660 μ) for the Beckman spectrophotometer Instead of a leukocyte suspension the same amount of duodenal juice (0 3 ml) was added to an incubation mixture which did not contain saponine With the exception of sample and reagent blanks each determination was run in duplicate and the pairs that did not agree within 10 of each other were rejected The values for the DAP activity were expressed as mg of phosphorus (P) liberated by 100 ml of juice A unit of enzyme activity is defined as the amount of enzyme required to catalyze the liberation of 1 mg of inorganic phosphate in 1 hour under the standard assay conditions The pH of the duodenal juice (7 8 to 8) which was measured with pHydrion paper was the most reliable guide to the position of the catheter tip which was also followed under fluoroscopy

RESULTS

The mean value for DAP activity of 100 ml of duodenal juice in the group of 10 control children was 4 119 ± 432 U (S D of 1 371) and in the 17 rachitic children 4 057 ± 291 U (S D of 1 202) The difference between these means was not significant ($p > 0$ 05) and the individual values are indicated in Fig 1

COMMENTS

Alkaline phosphatase of serum is heterogenous and comprises activities derived mainly from bone and to a variable extent *from the liver* and intestine (5 9) The isoenzymes produced

Acta Pædiat Scand 60 341–345, 1971

BAY B 5097 A NEW ORALLY APPLICABLE ANTIFUNGAL SUBSTANCE WITH BROADSPECTRUM ACTIVITY

Preliminary Clinical and Laboratory Experiences in Children

WALTER MARGET and DIETER ADAM

From the Children's Hospital University of Munich Germany

The antimycotic BAY b 5097 bis phenyl (2-chlorophenyl) 1 imidazolylmethane was first described in the summer of 1969 (1 2 3) Animal experiments as well as previous experience with one adult prompted us to start treatment with BAY b 5097 in an infant suffering from a life threatening Candida pyelonephritis and then to use it also in other children

We are observing an increasing incidence of infections with Candida albicans during broad spectrum chemotherapy among our patients and therefore urgently need a new drug to treat these children Subsequent to the combined use of Gentamicin with Carbenicillin or the Cephalosporines in infections of newborns as well as in children with suppression of the immune response induced by diseases or drugs systemic mycoses are increasing in our hospital Thus during the past year altogether 12 severe systemic mycoses were observed some of which seemed particularly suited for treatment with BAY b 5097 because of the clinically demonstrated unjustifiable side-effects of Amphotericin B (very poor general condition with retention of urea N) An attempt was also made to treat a case of toxoplasmosis with an unfavourable prognosis

MATERIAL AND METHODS

Clinical studies

The patients were 10 children aged from 5 months to 7 years (Table 1) All children suffered from severe chronic diseases (chronic pyelonephritis with anomalies of the urinary tract, vitiae cordis atopica dermatitis) Besides antibacterial chemotherapy Amphotericin B had been tried previously in 2 children with pyelonephritis and Nystatin locally in the atopical dermatitis All children had cross infections of Candida albicans No isolated strain was resistant to BAY b 5097 (personal communication of M Plempel) In some children we were able to find an increased antibody titre to the homologous strain of Candida albicans which otherwise is not demonstrable in infants Those urinary findings which showed more than 10^5 micro-organisms/ml and a simultaneous increase in leucocytes above 50/μl were considered as Candida albicans pyelonephritis

The 6 cases of pyelonephritis were treated with a dosage of 70 to 200 mg/kg/day The treatment lasted for 4 to 40 days.

Laboratory studies

The results of the serum and urine concentration found are based on thinlayer-chromatographic studies The substance can be isolated with organic solvents from urine blood or serum and can be prepared in the following manner Serum is precipitated twice with the same quantity of petroleum ether the petroleum ether is separated and concentrated for drying. The residue is put in chloroform and is ascendingly chromatographed on silica gel foils with chloroform as a fluent. The dry chromatogram is sprayed with 10% trichloroacetic acid heated for 2 3 min up to 100 C, the resultant yellow bands are scratched off the foil 2 ml of butylacetate and 3 ml of perchloric acid are added and measured at 475 nm in the photometer A 10 μg solution of the preparation is used as reference This is a method which mainly determines the metabolites of the substance By means of the agar-diffusion test with stainless steel cylinders according to the recommendations of Shadomy (4) for the testing of Amphotericin B and Hamycin, our Candida strain can be used to demonstrate the biologically active courses of the blood concentration of BAY b 5097 However we

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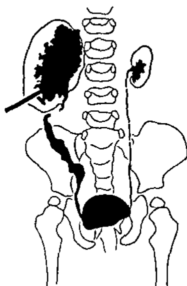


Fig 1 Drawing of a superprojected i v pyelogram and a mictioncystourethrogram of patient 1 *Right* atresia of the ureter and nephrostomy *Left* kidney atrophy

have not yet gathered sufficient experience to make reliable pharmacokinetic statements for children.

RESULTS AND DISCUSSION

In our opinion it is the child P R which deserves special attention He was treated in our hospital for the first time when he was in a hopeless condition (Figs 1 and 2) This child showed on the right side an ureterostoma after a hydronephrosis and an obstructive pyelonephritis After development of an urcterostoma the ureter became completely obstructed On the left side the i v pyelogram showed an abnormally small kidney The mictioncystourethrogram on the right side showed a reflux into a strongly dilated ureterstump and on the left normal conditions without reflux On the basis of this finding we were able to obtain urine separately from the right and the left kidney and to assess the germ counts per ml separately

In the beginning we did not know the compatibility of this new product in children and therefore we treated at first for 4 days i e as long as Candida albicans had been eliminated from the urine almost completely A relapse occurred after 22 days whereupon the drug was given in the same dosage for 11 days Again after discontinuation a renewed life threatening relapse occurred which could be controlled after a duration of treatment of 18 days with the same dosage and which led to the healing of the Candida infection In the subsequent period (2 years) the child had no further relapse and thrived normally

The serum concentrations in 2 patients are shown in Table 2 In the first hour it was 11 in the third hour 10–20 and in the sixth hour 13–25 μg/ml 10 hours after administration a clearcut decrease could be observed except in the child with burns on the seventh day of treatment. Excretion was relatively slight. Thus a 12 hours excretion of 10 15 mg or 7 05

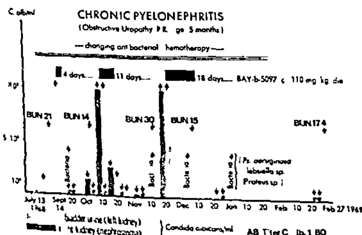


Fig 2 Candida albicans-pyelonephritis after various periods of treatment with BAY b 5097 in patient 1 Relapse after 4 days treatment, after 11 days treatment and 4 weeks interval without treatment a serious recurrence with increase in BUN Healing after 18 days of treatment.

Table 1 *Clinical summaries of ten patients treated with BAY b 5097*

Name Patient no	Age (years)	Diagnosis: Main diagnosis	Diagnosis: Infection due by C albicans	Diagnostic findings: Number of organisms in urine/ml[a]	Diagnostic findings: WBC in urine per μl	Diagnostic findings: Blood culture	Therapy BAY b 5097: mg/kg/day	Therapy BAY b 5097: Days	Results after treatments: Candida albicans findings in urine	Results after treatments: Side Effects	Results after treatments: Relapse	Notes
P R[b] 1	1	Obstructive uropathy	Chronic pyelonephritis (PN)	> 10^5	250	Neg	110–140 (2 × 80)	4/ 11/ 18	Neg some organ	None	44[c] see fig 1a 2	Candida albicans hemaggl AB titre 1 80
F C 2	4	Deep scalding 70 (grade II)	PN	> 10^5	160	Neg	120 (2 × 60)	9	Neg	SGOT 59 3 SGPT 40 8 Alk Phos 157 40 Weariness	No 30[c]	After discontinuing normalized Transaminases
S S 3	10/12	Meningo myelocele	Chronic PN	5 × 10^3	500–1 000	—	70	21	Neg	None	—	Further treatment
W T 4	1 6/12	Transv lesion of the cord	PN	> 10^3	80	—	100 (2 × 50)	17	Neg	None	No 5[c]	—
M P 5	3 7/12	Hydropyo nephrosis	PN	10^5	500	—	75	10	Neg	None	?	(Pat out of control)
H G 6	5	Atopic dermatitis	Candidiasis with positive feces and throat swab	—	—	Neg	100 (2 × 50)	38	—	None	Yes	5 weeks after discontinuing relapse
G U 7	2 9/12	Hydropyo nephrosis	PN	> 10^5	140	—	70	21	Neg	None	No 14[c]	
H A 8	9 9/12	Meningoencephalitis (Tbc)	Candida septicemia	—	—	+ + +	75–100	6	—	None	—	Exitus (Tbc) blood cult Candida neg
R A 9	4	Ventricular septum defect	Candida septicemia	—	—	+ + +	135 (3 × 45)	12	—	None	—	Further treatment
H T 10	11/12	Severe congenital Toxoplasmosis 2 relapses after Daraprime and Sulfonamides	—	—	—	—	150–250'	40'	—	(Slight weariness)	No 22[c]	Sabin's dye test before 1 4000 after treatment 1 4000 KBR before 1 20 after treatment 1 5

[a] Findings refer only to Candida albicans and not to other causative organisms of chronic pyelonephritis (Candida pyelonephritis as a mono-infection was never observed)
[b] Compare Figs 1 and 2
[c] Observed period after the end of treatment in weeks

Fig 1 Drawing of a superprojected i v pyelogram and a mictioncystourethrogram of patient 1 *Right* atresia of the ureter and nephrostomy *Left* kidney atrophy

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Fig 2 Candida albicans-pyelonephritis after various periods of treatment with BAY b 5097 in patient 1 Relapse after 4 days treatment, after 11 days treatment and 4 weeks interval without treatment a serious recurrence with increase in BUN Healing after 18 days of treatment

Table 2 *Concentrations of BAY b 5097 (Metabolite) in the serum of patients 6 and 2*

	Case 6		Case 2	
Time after administration (h)	Onset of therapy (1st day) twice 50 mg/kg/day (µg/ml)	Cont therapy (4th day) twice 50 mg/kg/day (µg/ml)	Onset of therapy (1st day) once 60 mg/kg/day (µg/ml)	Cont therapy (7th day) twice 60 mg/kg/day (µg/ml)
1	11			
3	17	10	11	20
4	18			
5		14		
6	16		13	25
7		13		
9		8		
10	14		6	24
11		6		
13		5		11
24			6	

mg could be determined in case 6, on the first to fourth day with a total ingestion of 1 g of the substance

In case 2, also receiving a total daily amount of 1 g a 24 hours' excretion of only 24 mg could be found As shown in Table 1, no relapses were observed after treatment of pyelonephritis had been discontinued, though the substance used for the treatment was a drug having a fungistatic effect only Furthermore, we treated a toxoplasmosis in a hopeless condition Previously, the child had been treated at an interval of several weeks twice with Daraprim and Sulfadiazine in a high dosage for 3 weeks After long term treatment with BAY b 5097, during which we administered up to 200 mg/kg daily, a probable healing could be observed which persisted until the time of writing (22 weeks)

In children with severe chronic diseases displaying, due to their disease an increased disposition to the demonstrated infection with Candida albicans we were able to carry out successful treatment with BAY b 5097 This leads to the conclusion that this substance has a high therapeutic effect in view of the susceptibility of the patients Blood concentrations of the metabolites show that the product is very well absorbed and shows a retarded excretion According to our studies it is still not clear how the drug is excreted, whether via the intestine or the bile or another route We were unable to demonstrate considerable amounts of the substance or its metabolites in the urine

In order better to evaluate the drug, further follow up of the clinical therapeutic results is necessary We must also understand the pharmacokinetics of biologically active substances and determine the mode of excretion The efficacy of the drug in infections with Candida in severely predamaged children is remarkable, particularly in view of its fungistatic action

Side-effects

In one child with a 70% second degree burn, showing renal damage and pyelonephritis we could observe a cumulative effect of the product (Table 2 case 2) This concentration led to a remarkable fatigue and an increase of the transaminases SGPT and SGOT These symptoms disappeared immediately after discontinuation of the drug

SUMMARY

BAY b 5097 bis phenyl (2-chlorophenyl) 1 imidazolylmethane, is a fungistatically acting new antimycotic which was used in 10 chil-

dren in a dosage of 70–200 mg/kg/day usually in two doses Six children suffered from a Candida albicans pyelonephritis 2 from Candida septicemia, 1 from atopic dermatitis with candidiasis and 1 from congenital toxoplasmosis All children responded to the therapy The possible successful treatment of a hopeless case of toxoplasmosis leads to the assumption that there might be an effect on protozoa

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CASE REPORT

THE CUTIS VERTICIS GYRATA AND MENTAL RETARDATION SYNDROME IN A 4-YEAR OLD BOY

J PALO and M IIVANAINEN

From the Department of Neurology University of Helsinki Helsinki and the Research Department Rinnekoti Institution for the Mentally Retarded Majalampi Finland

The combination of cutis verticis gyrata (corrugated or gyrate appearance of the scalp) and mental retardation has been published in more than one hundred cases A maldevelopment of skull and/or brain is encountered in most and probably all patients (4) Certain evidence points also to an endocrine disorder (1 2)

Very few female patients with the cutis verticis gyrata and mental retardation syndrome have been reported In addition, the scalp anomaly has been observed in patients who have reached or already passed their puberty The only exception was an 11 year old girl (5) The rare onset before puberty and the disproportion between the sexes are the most widely quoted criteria to support the theory that cutis verticis gyrata is caused by an endocrine disorder We would like to present a 4-year old boy with cutis verticis gyrata and mental retardation He is the youngest patient ever seen with this rare condition

CASE REPORT

The patient was born August 30 1966 The father mother two siblings (born in 1960 and 1964) and all other members of the family were healthy The pregnancy was normal The delivery was 3 weeks overdue and labour was induced The birth weight was 3 980 g and length 51 cm The patient scored 10 points in the Apgar scale The head circumference was small 33 5 cm and the parietal bones were overlapping in the sagittal suture but this was within the normal limits At the age of 1 month it was observed that the head circumference did not increase and at the age of 6 weeks the child had his first convulsions At 3 months he had status epilepticus and was hospitalized The EEG recording consisted of high and slow (2-6 c/s) waves mixed with frequent spikes and sharp waves The finding was somewhat typical for hypsarrhythmia although the very high number of spikes and the localization of the abnormal waves to the right side did not quite correlate to the diagnosis The convulsions were also different from infantile spasms Other clinical findings were microcephaly spastic tetraplegia and unspecific generalized aminoaciduria The scalp was normal except for dandruff and erosions at the age of 5 months The patient was unresponsive to visual and auditory stimuli A treatment with ACTH was started and given during 6 weeks at the age of 5–6 months During that period only a few mild convulsions were seen but afterwards the patient had a typical Cushing face The EEG showed no hypsarrhythmia but there were some bursts of sharp and slow waves more on the left He was then on a constant anti epileptic medication but had nevertheless occasional grand mal seizures The cutis verticis gyrata was observed in connection with a cutaneous infection of the scalp

At the age of 3 years and 2 months the patient was unable to sit or walk The head was asymmetric with a circumference of 39 5 cm Four folds were easily palpable and visible in the left parietal region of the scalp (Fig 1) Micrognathia short neck and marmorated skin were also observed The patient was probably blind with a horizontal bilateral continuous nystagmus and an outward deviation of the left eye He seemed to react to strong sounds The marked loss of hearing ability was confirmed with the evoked



Fig 1 Four cutis verticis gyrata folds are visible on *the left parietal area of the scalp*



Fig 3 Gas encephalography showing enlarged ambient cisterns The brain stem is hypoplastic Note the markedly asymmetric skull.

potential examination. He had thoracic scoliosis and mild contractures in both knees All the extremities *showed some spasticity during active movements al* though they felt quite hypotonic during rest. Babinski sign was positive on both sides The patient reacted to pain He had no bladder or bowel control and could not eat or clothe himself His I Q was well below 10 X ray showed asymmetric small cranium where the left side was larger than the right side A marked asymmetric macroventriculy (Fig. 2) was observed in pneumoencephalography together with a hypoplastic brain stem (Fig 3) and cerebellum (Fig. 4) The width of the third ventricle was 16 mm

Routine blood urine and cerebrospinal fluid tests showed no specific changes. Chromosome analysis was normal An ACTH loading test was also performed Serum cortisol value was 42 0 $\mu g/100$ ml before the intramuscular injection of 20 I U of natural ACTH (Cortrophine®) 77 7 $\mu g/100$ ml after 1 h 75 $\mu g/100$ ml after 2 h, and 53 1 $\mu g/100$ ml after 3 h of the injection Thorn s test confirmed the normal adrenocortical function (31 blood eosinophils/mm^3 before the test and 6/mm^3 after 4 h of the intramuscular injection of 20 I U of natural ACTH)

DISCUSSION

Although maldevelopment of skull and brain is very *frequent in the cutis verticis* gyrata and



Fig Gas encephalography showing asymmetric, markedly enlarged lateral ventricles. The frontal horn and cella media of the left lateral ventricle are larger than those of the right one



Fig 4 Gas encephalography showing hypoplastic cerebellum and brain stem in lateral projection.

mental retardation syndrome (4), the origin of the scalp malformation remains obscure Endocrinological factors may have some etiological significance, this hypothesis is supported by the fact that most patients are males and that only 2 paediatric cases have thus far been diagnosed The present patient was given ACTH in early infancy It can be speculated that this treatment either caused cutis verticis gyrata or at least had some contributory effect to its formation However, there were no known cases of cutis verticis gyrata among 70 children treated with ACTH because of infantile spasms at the Children s Hospital in Helsinki Congenital existence of the malformation is, of course, more obvious The scalp may become more easily folded, when the skull has a small circumference (3) The encephalographic findings and the existence of several malformations support the theory of prenatal origin of the disorder, and thus the hypothesis of the secondary nature of possible endocrinological abnormalities At present the patient seems to have a normal pituitary and adrenocortical function despite of the wide third ventricle This is in agreement with the results obtained in a larger series where no consistent endocrinological abnormalities could be observed (4)

The 4 year-old boy with the cutis verticis gyrata and mental retardation syndrome described above is an interesting example of a syndrome that was long believed to appear only after puberty The development of the scalp anomaly will be followed

SUMMARY

A 4 year old profoundly mentally retarded boy with cutis verticis gyrata is described He had four folds on the left parietal region small asymmetric head, micrognathia short neck, marmorated skin, thoracic scoliosis, spastic tetraplegia and epilepsy Encephalography revealed marked asymmetric macroventricuły with hypoplastic brain stem and cerebellum Thorn s test and corticotrophin stimulation test gave normal responses The boy is the youngest patient with the cutis verticis gyrata and mental retardation syndrome described in the literature The numerous malformations of the patient strongly support the theory of prenatal origin of the cutis verticis gyrata and mental retardation syndrome

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CASE REPORT

A CASE OF TUBEROSE SCLEROSIS IN THE NEWBORN

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The first record of sclerosis of the cerebral cortex accompanied by myomata of the heart was made by Von Recklinghausen in 1862 and later by H Vogt (4) The clinico-pathological syndrome which we know today as the tuberose sclerosis complex has been summarised by H Globus (3) in recent times

This consists of an association of cerebral sclerosis with hamartomata of the skin heart, kidney bone and lung and is clinically recognised by the association of adenoma sebaceum and epilepsy The cardiac hamartomatous lesions are usually called rhabdomyomata which are commonly present as multiple nodules throughout the substance of the heart.

Batchelor & Mann (2) showed that at least 50 of cases of cardiac rhabdomyoma were associated with cerebral sclerosis whilst about 7 of those with cardiac lesions died in the neonatal period

CASE REPORT

The mother of the affected infant was a 25 year-old primigravida, induced and delivered by forceps of a live male infant weighing 2 346 g under the care of Dr B D Corner The onset of respiration was spontaneous but then became irregular and gasping. Intubation and intermittent positive pressure respiration failed to maintain adequate oxygenation the cardiac rate slowed and a harsh pansystolic *murmur* was heard The infant died aged 1 hours.

Post mortem findings

The body was that of a mature cyanosed male infant of 47 cm crown-heel length. The right pleural cavity contained gas under pressure indicating the presence of a tension pneumothorax The pericardium contained an excess of clear serous fluid.

The heart was grossly distorted by the presence of several tumour masses within its substance the largest being 6 cm in diameter in the intraventricular septum There was another smaller lesion 1 5 cm *in diameter* situated just below the valve (Fig 1) Many other small nodules were present throughout the ventricular myocardium (Figs 1 and 2) Both lungs were small and the left lung was completely unexpanded whilst expansion was only partial within the right The kidneys showed a few small cysts measuring up to 0 4 cm in diameter Other organs throughout the body appeared to be normal

Histology

Sections of the renal cysts showed them to be lined by a rather hypertrophic tubular type of epithelium

Sections from the cardiac tumours were fairly typical of the so-called Rhabdomyoma" consisting of a loose network of thin striated muscle grossly vacuolated by glycogen deposits to give an open mesh appearance within the cytoplasm of the swollen myocardial cells (Fig 2)

Central nervous system

At first, examination of the brain showed no appreciable abnormality but after fixation although the gyral pattern was normal many large irregular rubbery tubers could be seen and palpated in the gyri of both cerebral hemispheres *Coronal slicing of the* specimen revealed irregular thickening and distortion of the cortical ribbon wherever a tumour was present There were also *numerous* firm pale nodules seen throughout the central white matter of each hemisphere (Fig 3) Similar nodules were also seen beneath the ependyma of the lateral ventricles some of these projected into its cavity especially into that of the anterior horn on the right side.

No heterotopic nodules could be recognised within the brainstem or within the substance of the cerebellum Sections were taken from numerous areas of the brain including the cerebral cortex through to the lateral ventricles, the basal *grey nuclei* the brainstem and cerebellum

Histologically many of the typical neuropathological *features of tuberose* sclerosis were present The tubers within the cortex consisted of aggregates of grossly

mental retardation syndrome (4), the origin of the scalp malformation remains obscure Endocrinological factors may have some etiological significance this hypothesis is supported by the fact that most patients are males and that only 2 paediatric cases have thus far been diagnosed The present patient was given ACTH in early infancy It can be speculated that this treatment either caused cutis verticis gyrata or at least had some contributory effect to its formation However, there were no known cases of cutis verticis gyrata among 70 children treated with ACTH because of infantile spasms at the Children s Hospital in Helsinki Congenital existence of the malformation is, of course, more obvious The scalp may become more easily folded, when the skull has a small circumference (3) The encephalographic findings and the existence of several malformations support the theory of prenatal origin of the disorder and thus the hypothesis of the secondary nature of possible endocrinological abnormalities At present the patient seems to have a normal pituitary and adrenocortical function despite of the wide third ventricle This is in agreement with the results obtained in a larger series where no consistent endocrinological abnormalities could be observed (4)

The 4 year old boy with the cutis verticis gyrata and mental retardation syndrome described above is an interesting example of a syndrome that was long believed to appear only after puberty The development of the scalp anomaly will be followed

SUMMARY

A 4 year old profoundly mentally retarded boy with cutis verticis gyrata is described He had four folds on the left parietal region small asymmetric head, micrognathia, short neck, marmorated skin, thoracic scoliosis, spastic tetraplegia and epilepsy Encephalography revealed marked asymmetric macroventriculy with hypoplastic brain stem and cerebellum Thorn s test and corticotrophin stimulation test gave normal responses The boy is the youngest patient with the cutis verticis gyrata and mental retardation syndrome described in the literature The numerous malformations of the patient strongly support the theory of prenatal origin of the cutis verticis gyrata and mental retardation syndrome

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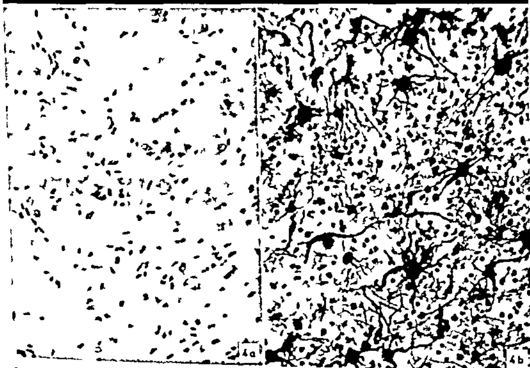


Fig 3 Coronal slice showing tubers in cortex and subependyma nodules.

Fig 4 (*a*) Heterotopic nodule of giant astrocytes. H&E, 100 (*b*) A similar area after silver impregnation SCA, 100

bnormal astrocytes causing disturbance of lamination but not associated with any excess of glial fibre production Many of the cells constituting these tubers were bi or tri nucleate as were similar collections of cells within the substance of the white matter which were partly astrocytic and partly neuronal in

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2

Fig 1 Tumour obstructing pulmonary outflow tract

Fig 2 Rhabdomyoma showing vacuolated cardiac muscle cells H&E ×200



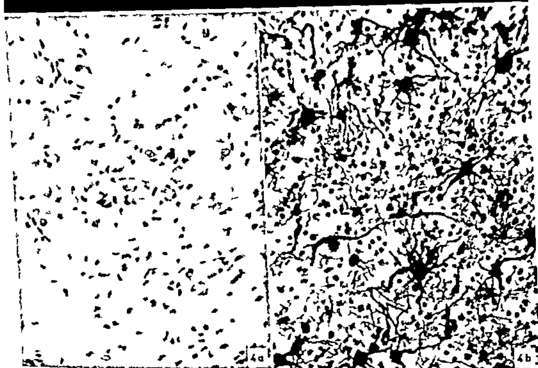


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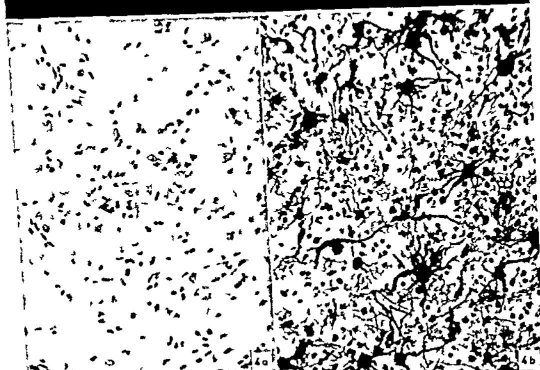


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Fig 5 Fibroblasts and glial cells in a subependymal nodule PTAH × 100

nature (Fig 4) The subependymal nodules were of the typical fibro cellular type consisting of large round or oval cells *intermingled with leashes of glial fibrils* originating from the interspersed fibrillary astrocytes (Fig 5) No heterotopic nodules could be seen in the brainstem or cerebellum neither were there any giant Purkinje cells demonstrated within the cerebellar cortex

DISCUSSION

This infant presented as a resuscitation problem the immediate cause of death being disturbance of cardiac function due to obstruction of the pulmonary outflow tract by the presence of the numerous and large cardiac tumours Because of the typical lesions in the brain and the rhabdomyomata of the myocardium this infant must be considered as a random dominant case of Tuberose sclerosis In this case there is no family history of any similar disorder

The extent of the lesions in the brain was also unusual and presumably indicates a more complete expression of this variable disorder of histogenesis Alzheimer (1) than is usually seen

The heavy gliosis, normally seen in this condition was not seen in this case, but death occurred early in the neonatal period and reactive changes would not have had time to occur The little gliosis that was seen could well have occurred during intrauterine life

SUMMARY

This paper describes an infant with tuberose sclerosis which died in the neonatal period from cardiac failure associated with multiple Rhabdomyomata of the heart

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CASE REPORT

ECTOPIC SPLEEN IN AN Rh INCOMPATIBLE INFANT

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Ectopic spleen is a rare condition in the new born period The diagnosis is to be considered in the presence of a tumoral mass in the abdomen of the newborn in the absence of a detectable spleen at its usual site This condition has to be kept in mind especially when an abdominal mass is present in a newborn with Rh incompatibility where splenomegaly is expected but no spleen is palpable at its normal position

The following is a case in which an ectopic spleen was diagnosed preoperatively in a newborn in the presence of Rh incompatibility with a relatively high level of conjugated bilirubin.

CASE REPORT

The mother was a 38 years-old Jewish woman of Iraqi origin. Her present pregnancy was the seventh and she has given birth to six children The blood group of the mother was found to be B Rh negative No signs of hemolytic disease were observed in the first six children although all of them were Rh positives

The present pregnancy developed normally and the titer of the indirect Coombs test rose to 1 64 2 weeks before the delivery A male baby of 4 000 g was delivered spontaneously in the 40th week of gestation Three hours after delivery he became pale and a slight degree of jaundice developed The liver was palpable 4 cm below the costal margin No spleen was palpable in left upper quadrant A firm oval mass about 5 6 cm was found in the left lower quadrant (Fig. 1) It had a smooth surface and was freely movable about 2 cm in all directions No pain seemed to be elicited in the infant on examination of the mass A provisional diagnosis of Ectopic spleen was made

Laboratory findings The total cord blood bilirubin was 4 5 mg/100 ml from which the direct binding fraction represented 1 5 mg/100 ml Hemoglobin 12 0 g/100 ml Blood group was found to be B Rh positive and the direct Coombs test was positive Chest and abdominal X rays showed no pathological findings. 20 hours after birth the hemoglobin was found to be 11 g/100 ml. The leukocyte count was 34 700/mm with 40% normoblasts and the reticulocyte count was 13% The bilirubin rose to 11 mg/100 ml with 3 mg/100 ml direct binding bilirubin The urine was found positive for bilirubin

On the 5th day the hemoglobin level went down to 9 g/100 ml and the total bilirubin reached 32 mg/100 ml, of which 22 mg/100 ml was of conjugated form An exchange transfusion was performed mainly to arrest the increasing anemia 400 ml of B Rh negative blood was used for the exchange In spite of another rise in the total bilirubin level, a second exchange transfusion was not considered to be necessary since the hemoglobin was stable at about 13 g/100 ml and the indirect bilirubin was found to be only one third of the total Gradually the jaundice subsided and at 2 weeks of age a slight decrease in the volume of the mass in the left lower quadrant was noted After an intravenous drip pyelography and barium enema were performed and found normal the possibility of the mass being an ectopic spleen was investigated A scintigram was performed after injecting ^{51}Cr labeled autologous red blood cells (Fig. 2) This revealed the presence of a round mass about 6 cm diameter in the left abdomen but neither in the position of a normal spleen, nor where the mass was previously palpated but midway between these two points.

Exploratory laparatomy was performed at the age of 6 weeks. An intraperitoneally situated ectopic



Fig 1 Patient after birth without palpable spleen at usual site but with round mass in lower left abdomen

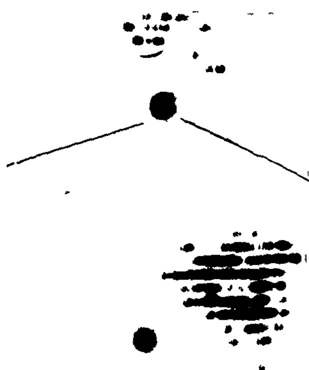


Fig 2 The scintigram after injection of ^{51}Cr labeled autologous red blood cells revealed a mass midway between the palpated tumor and the usual position of an enlarged spleen

spleen with an infarcted area at its upper pole was found in the left iliac fossa on a long partially torsioned vascular pedicle

Normal ligaments connecting the spleen to its neighboring organs were not found although the spleen was bound to the ileum by numerous adhesions The gallbladder and the extrahepatic bile ducts appeared normal and no accessory spleen was seen Splenectomy was performed in view of the ectopic infarcted spleen the adhesions and the torsion of its long pedicle

The ectopic spleen was enlarged weight 110 g and the infarcted upper part was well delineated from the remainder (Fig 3) At microscopy apart from the large infarcted area the remainder of the splenic tissue was normal with an increased number of normoblasts and an excess of iron pigment

The post operative course was uneventful The baby was discharged a month later with a thrombocyte count of 800 000/mm³ No serious infections occurred in the infant during a 24 months follow up period

DISCUSSION

Wandering spleen is a rare condition usually found in patients with splenomegaly and laxity of the abdominal wall In a series of 1 437 cases of splenic diseases seen over a period of 10 years not a single case of wandering spleen was reported (6) The finding of an ectopic spleen in a newborn infant is an even greater rarity and possibly related to developmental defects of the fixation of the spleen in its nor



Fig 3 Section through the enlarged spleen with th infarcted area at the upper pole

mal position Apart from this condition involving the sites of the spleen varying numbers of accessory" or supranumerary spleens are much more common Their most frequent sites are the hilum of the spleen gastrosplenic ligament, and the greater omentum and rarely the scrotum liver and pancreas (2)

The spleen appears in embryos of about 10 mm crown rump length as a condensation of *mesodermal cells in the dorsal mesogastrium* The enlarging spleen later projects through the left leaf of the dorsal mesogastrium and the mesentery dorsal to it rotates backwards to the left and fuses with the peritoneum of the posterior abdominal wall Dorsal to the spleen the mesentery forms the lienorenal ligament and ventral to it the mesentery forms the gastrosplenic ligament (3) It has been suggested that a poorly developed gastrosplenic ligament allows excessive splenic mobility (5) Increased mobility of the splenic hilus may also be due to the incomplete fusion of the mesogastrium posteriorly while at the same time the lienorenal ligaments is not formed (7)

Although the spleen enlarges in fetuses severely affected by Rh incompatibility only in the last third of the pregnancy its increase in weight and volume could at least theoretically influence its location should some earlier developmental defect preexist In the present case the spleen at operation was found close to the bulk of the ileum with numerous adhesions between them and since no suspensory ligaments of the spleen were found it must have been ectopic

This hypothesis is supported by the surprising similarities with the case reported by Daum et al of an erythroblastotic newborn with an enlarged spleen discovered in the right lower abdomen and no suspensory ligaments found at operation (1) The features of the two cases are listed in Table 1

The finding of a high level of conjugated bilirubin in some cases of erythroblastosis fetalis is known (4) There are no evident links between ectopic spleen and the obstructive type of jaundice observed in our patient Nevertheless it is intriguing that the same association existed in the only additional case of ectopic spleen in erythroblastotic newborn found in the literature

Table 1 *Similarities of features between the present case and the patient reported by Daum and coworkers*

Essential data	This case	Daum s case
1 Hemolytic disease due to Rh incompatibility	Present	Present
2 Exchange transfusion performed	Once	Twice
3 Obstructive type of jaundice	Present	Present
4 Spleen at normal site	Absent	Absent
5 Mass in lower abdomen (ectopic spleen)	Present (left)	Present (right)
6 Suspensory ligaments of spleen	Not found	Not found
7 Partial infarction of ectopic spleen	Present	Present

SUMMARY

A case of a newborn with a Erythroblastosis fetalis and obstructive type jaundice with an abdominal tumor is presented As the jaundice was found to be elicited by Rh incompatibility the absence of a palpable spleen at the normal site suggested the possibility of the abdominal mass being an ectopic spleen This diagnosis was confirmed at operation The possibility of a preexisting developmental defect is elaborated upon

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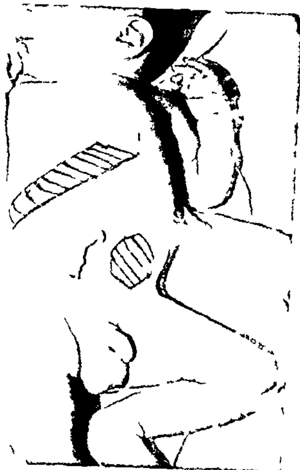


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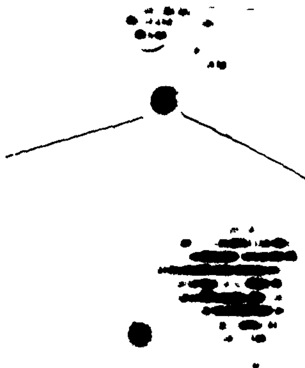


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Fig 3 Section through the enlarged spleen with the infarcted area at the upper pole

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CASE REPORT

CUTIS LAXA ASSOCIATED WITH SEVERE INTRAUTERINE GROWTH RETARDATION AND CONGENITAL DISLOCATION OF THE HIP

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From the Department of Paediatrics Orthopaedics and Pathology of the Beilinson Hospital Petah Tikva and the Tel Aviv University Medical School Israel

Congenital cutis laxa is a rare familial disorder (7) Characteristically the skin hangs loose in pendulous folds giving the patient an aged appearance As this is a disease of the connective tissues other organs may also be affected Therefore apart from the cosmetic disturbance the condition may cause lethal generalised illness depending on the organ affected (9 10 12)

The purpose of this paper is to report two siblings with congenital cutis laxa associated with severe intrauterine growth retardation (I U G R) and congenital dislocation of the hip (C D H)

CASE REPORT

Case 1 C N

This infant was the second child born to a 26-year old *asthmatic mother and a 32 year old healthy* father The parents were first cousins The duration of pregnancy was 4? weeks and the delivery was normal

Her birth weight was 2 150 g, height 43 5 cm and head circumference 32 cm On examination she was noted to have a very dry wrinkled skin with large tortuous veins (Fig 1) The face was wrinkled and the eyes were sunken The ears were low set. Both fontanels were large the anterior approximately 6 cm wide and the posterior 3 cm wide with the sutures markedly separated The palate was high arched A transverse palmar crease was noted on the right hand On examination of the hips, a click" was elicited in the left hip joint and C D H was confirmed by X ray Treatment with conservative measures was unsuccessful

At the age of 1 year she weighed 6 300 g (50th percentile for 4 months) her height was 68 cm (50th percentile for 6 months) She spoke a few words and her responses were within normal limits for her age A marked redundancy of skin was noted particularly of the abdomen hands and feet, with distended veins Hyperlaxity of all the joints was found

Blood and urine examinations at the age of 1 year were all normal except that on electrophoretic fractionation of the serum an elevated level of alpha 2 globulin was found Cytogenetic studies showed a *normal female karyotype*

At the age of 14 months open reduction of the left hip with limbectomy was successfully performed and a skin biopsy was taken

Pathological report (Fig 2) On histological examination of the skin using Weigert s elastic stain the elastic fibres were noted to be decreased in amount especially in the papillary region with degenerative changes Some of the fibres were fragmented, others were either shorter or longer as well as thinner than normal fibres Control sections of the skin of the thigh of several healthy infants showed none of these changes.

The pathological changes were compatible with the diagnosis of cutis laxa.

Case 2 C T

This is the older sister of case 1 She was born following an otherwise normal pregnancy of 36 weeks duration weighing 1 400 g At the age of 4 months the infant was referred to the orthopaedic department for a suspected right C.D.H which was confirmed by X ray She was treated with conservative measures without any *improvement. At the age of 1 / years,* open reduction of the right hip with limbectomy

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Fig 1 Patient C N at the age of 2 days showing wrinkled skin with dilated veins on abdomen

and derotation osteotomy of the femur were successfully performed

She was first examined in the paediatric clinic at the age of 4 years Her weight was 11 700 g (50th percentile for 2 years) her height was 97 cm (10th percentile) and her head circumference was 45 cm (50th percentile for 1 year) There was marked redundancy of the skin with distended veins She had increased amounts of hair over her entire body Hypertelorism with bilateral epicanthus was noted Her palate was high and the ears were low set Hyperextensibility of the joints was found especially of the knee joints The wound of the operation on the right hip had healed Her development was slow in all respects

On ophthalmological examination coloboma of the macula was found in both eyes as well as severe myopia

DISCUSSION

The clinical diagnosis of congenital cutis laxa (chalazoderma dermatomegaly, dermatolysis or primary elastolysis) can usually be made by the typical skin findings The loose pendulous skin gives the affected person an aged appearance and in children it is suggestive of a suit of skin too large for the owner The disease is rare Goltz et al (7) reviewed the literature until 1965 They found 19 cases and added 2 of their own Subsequently a further 10 infants with this disorder have been reported (1, 2, 3, 5 9, 10, 11, 12) In most of the cases reported the birth weight of the infants was normal It appears to be more common in females than in males Involvement of connective tissues of internal organs may result in pulmonary emphysema with right heart disease (7, 9 12), gastrointestinal and urinary tract diverticulosis, vaginal and rectal prolapse, and the occurrence of a deep voice (7) Other findings include pulmonary artery stenosis (10) degenerative changes in the cornea (2), with severe mental retardation (3) In contrast to the Ehlers Danlos Syndrome where hyperlaxity of the ligaments with instability of the joints are usual features in cutis laxa no hyperextensibility is usually found (8), although this was noted in an infant reported by Debre (4)

In 1942 Fittke (6) reported a female infant born at term weighing 2 275 g with a birth length of 46 cm (Table 1) No consanguinity was known At birth the skin was noted to be redundant At the age of 10 ¹/ months the diagnosis of cutis laxa with right C D H was made



Fig 2 Skin biopsy in patient C N The amount of elastic tissue is decreased in the upper dermis ×400

Table 1 *Clinical findings in reported patients of cutis laxa with I U G R and C D H*

Case	Sex	Period of gestation	Birth weight (g)	Birth length (cm)	Consanguinity	C D H	Hyperlaxity
Fittke 1942 (6)	F	Term	2 275	46	None known	Rt hip	?
Bittel Dobrzynska 1964 (1)							
Case 1	F	Term	2 500	—	None	Bilateral	Yes
Case 2	F	Term	2 000	44	None	Bilateral	Yes
Case 3 (Sibling of 2)	F	Term	1 750	43	None	Bilateral	?
Reisner et al.							
Case 1	F	42 weeks	2 150	43 5	Yes	Lt hip	Yes
Case 2 (Sibling of 1)	F	36 weeks	1 400	—	Yes	Rt hip	Yes

No similar cases were reported until 1964 when Bittel Dobrzynska & Siniecki (1) reported 3 cases The pertinent details are given in Table 1

These 4 cases and our 2 infants have a number of findings in common (Table 1) They are all female infants born after a full term or post term pregnancy except for our case 2 All their birth weights were usually far below the 10th percentile for their period of gestation In those infants in whom the birth length was known this was also under the 10th percentile for gestation All had congenital dislocation of either one or both hips and in at least four of the six hyperlaxity of some joints was mentioned

These 6 infants appear to present a syndrome different from all the other reported cases of cutis laxa They are all female patients with I U G R both in weight and length Apart from the typical skin manifestations they present with hyperextensibility of their joints associated with C D H The mode of inheritance appears to be as an autosomal recessive gene The marked degree of I U G R may be indicative of a more severe form of the disease which might be lethal in the male foetus

SUMMARY

Two female siblings with cutis laxa are reported Both these infants showed marked intrauterine growth retardation hyperlaxity of the joints with congenital dislocation of the hip Four similar female infants have been reported in the literature The mode of inheritance appears to be as an autosomal recessive gene The marked degree of intrauterine growth retardation may be indicative of a more severe form of the disease which might be lethal in the male foetus

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LETTER TO THE EDITOR

To the Editor

Recently an article by O Senki on the inheritance of osteogenesis imperfecta congenita was published in your journal (Case Report Osteogenesis Imperfecta Congenita in One of Twins O Senki *Acta Pædiat Scand 59* 340–342 May 1970) Two cases were reported each concerning a set of twins in which one twin was afflicted with the disease and the other twin was not In each case only one of the parents was affected (the mother in Case 1 the father in Case 2) Although the twins were like sexed in both cases investigations to determine zygosity were not carried out in view of the fact that in both cases the twins were markedly dissimilar in appearance it does not seem feasible to propose monozygosity merely on the basis of sex.

Osteogenesis imperfecta congenita is generally considered to be inherited as an autosomal dominant trait with considerable variation in degree of expressivity The author of the twin studies suggests that the affected parents (mother in Case 1 father in Case 2) are heterozygous carriers and the condition in the babies was transmitted as a recessive characteristic Assuming that the affected parents were heterozygous carriers the other parent in each case was reported to be normal In such a case it seems unlikely for the condition to be transmitted as a recessive but rather it appears to be a case of autosomal dominant inheritance

Considering the lack of information regarding the parental genotype and the absence of any zygosity investigations I find it impossible to suggest transmission as a recessive characteristic

Patrick Sweeney
Senior Medical Student
St. Louis University School of Medicine
St. Louis Missouri

The Editor has asked Dr Senki to comment on this letter

To the Editor

Mr Sweeney raises three points namely that (1) investigations were not carried out (2) it does not seem feasible to propose monozygosity merely on the basis of sex and (3) that mode of inheritance is one of autosomal dominant.

In reply to the above criticism I would state that investigations were carried out but were incomplete because of sudden death in one twin of each pair For this reason, it was not possible to establish zygosity and they were labelled like sexed twins instead of mono- or dizygotic twins The parents of the second pair were available for clinical examination and only the father showed evidence of the disease The haemoglobin genotype in both parents were AS as well as the surviving twin. This information by itself does not confirm or disprove the point that the condition was transmitted as a recessive characteristic However if complete investigations had been possible and it could be shown that the twins though of the same sex were dizygotic this will be strong evidence in favour of an autosomal dominant transmission.

As I indicated there had been some uncertainty about the mode of inheritance in the reported cases and this is why I made the plea for a more detailed family studies investigation and reporting of cases particularly in the African population

Olumuyiwa Senki M D
Clinical Affiliate Fellow

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PROCEEDINGS OF PÆDIATRIC SOCIETIES

EUROPEAN SOCIETY FOR PÆDIATRIC GASTROENTEROLOGY

Meeting in Lund August 22–24 1970

Margot Shiner (London) & D H Shmerling (Zurich) *Ultrastructural changes after gluten*

The response of the jejunal villous epithelial cell and its subepithelial tissues to a single dose of gluten was studied with the electron microscope in a coeliac child treated with a gluten free diet for 3 years and compared with that of a non-coeliac child Twenty four hours after gluten administration the epithelial cells of the coeliac patient showed no abnormality but the subepithelial basement membrane was thickened the capillary endothelium showed swelling and there was an increase in lymphocytes eosinophils and plasma cells in the subepithelial connective tissue The ultrastructural changes in the non-coeliac patient 24 hours after gluten were entirely confined to the epithelial cell itself and consisted of large vacuoles surrounded by smooth membranes containing fibrous and various other unidentifiable material The absence of an intracellular reaction following gluten in the coeliac child could be due to an ineffectiveness in walling off exogenous material such as gluten and may be of importance in allowing antigenic material to pass through the cell in the coeliac patient but not in the non coeliac patient thus setting up immunological reactions in the subepithelial connective tissue and nearby capillary endothelium

E Savilahti J K Visakorpi & P Pelkonen (Helsinki) *Morphological and immunohistochemical findings in small intestinal biopsy in children with IgA deficiency*

The coexistence of immunoglobulin A deficiency and intestinal damage has frequently been reported We have investigated whether intestinal damage is present in children with isolated IgA deficiency Sixteen children over 2 years of age were found to lack serum IgA Small intestinal biopsy was performed on these children

Only one of the patients was admitted to hospital because of gastrointestinal symptoms and malabsorption was suspected in another because of severe iron deficiency anaemia Small intestinal biopsy revealed total villous atrophy in both The later clinical course was compatible with coeliac disease In the remaining 14 the morphology of the small intestinal mucosa was normal and in the 8 tested absorptive function was normal However all 16 patients had precipitating antibodies to cow s milk

Direct immunofluorescent studies on both small intestinal and rectal biopsy specimens were performed in 13 patients in another 2 only one of these specimens was available No positive fluorescence with anti IgA antiserum was seen in any specimen from the 14 patients In one case the number of IgA-containing cells was high in both small intestinal and rectal specimens but even in this case IgM-containing cells predominated This was the only patient whose serum contained trace amounts of IgA (0 5–2 0 mg/100 ml) Faint staining of interepithelial spaces with anti IgM antiserum was evident in the lamina propria of small intestinal specimens and this was more pronounced in the 2 cases with villous atrophy In rectal specimens clear fluorescence with anti IgM antiserum was seen in the apical portions of the rectal glandular epithelium

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ON EXPERIMENTAL TREATMENT OF PREMATURE ICTERIC INFANTS WITH OROTIC ACID

In the January Issue of this journal (*Acta Pædiat Scand 60* 1, 1971) Kintzel, Hinkel and Schwarze report that treatment of premature icteric infants resulted in a decrease in the serum bilirubin level Orotic acid is a precursor of UDPGA the co-enzyme of bilirubin conjugation

Preliminary experiments in our laboratory have shown that orotic acid competes with bilirubin for the binding site on albumin The displacing effect is stronger than that of salicylic acid Until the opposite has been proved it would thus be possible to explain the decrease of serum bilirubin concentration as an effect of such displacement It may be argued that according to the authors the treated infants *showed no detrimental effects while displacement of bilirubin from the binding to albumin* would most likely precipitate kernicterus in several children It may still be justified, however, to consider this possible side effect and we fully agree with the authors when they state that it is necessary to examine the question of side effects still more carefully before orotic acid is routinely administered to prevent premature infants from contracting hyperbilirubinaemia

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The morphological study indicates that the two uncommon conditions, coeliac disease and IgA deficiency, coexist with much higher than random frequency The presence of precipitating antibodies to cow s milk indicates that the integrity of the small intestinal mucosa is in some way disturbed in IgA deficiency

The immunofluorescent study verified the predominance of IgM containing cells in morphologic il normal intestinal mucosa demonstrated by others in IgA-deficient coeliac patients The study also suggests that IgM is secreted onto the intestinal mucosa

S Auricchio (Naples) F Ciccimarra (Naples), J Jos (Paris), F Rey (Paris), L Moauro (Naples) & J Rey (Paris) *Studies on intestinal digestion of starch in children with congenital deficiency of sucrase and isomaltase activity*

The analysis of the carbohydrates of intestinal juice after a test meal containing amylopectin in 5 patients, as compared with three normal controls, shows that the salivary and pancreatic alpha amylolysis of amylopectin in the proximal jejunum is normal After the first year of life, intestinal juice contains glucose, maltose, maltotriose and low and high molecular weight branched dextrins The intraluminal digestion of the polysaccharide is, on the contrary incomplete both in the patients and in the controls younger than 12 months of age, probably as a consequence of the physiologically low levels of alpha amylase activity The maltotriose oral tolerance test is normal in a three year old patient, whereas it causes slightly fermentative diarrhoea and a small increase of blood glucose in a 6 month old patient The mucosal glucamylolytic digestion of starch is impaired in intestinal biopsies of 9 patients probably as a result of the deficiency of glucamylase activity of the sucrase isomaltase complex

These results suggest that starch malabsorption in congenital sucrase isomaltase deficiency is due to defects in some of the mucosal enzymes involved in the digestion of starch and of the products of alpha amylolysis of the polysaccharide

J Schmitz, M Odievre & J Rey (Paris) *Fructose free diet effects on intestinal a glucosidase activities in hereditary fructose intolerance*

Intestinal *a* glucosidase activities of 9 patients affected by hereditary fructose intolerance have been compared with those of 8 normal children The patients were kept on a fructose free diet for at least 15 months Biopsy specimens were obtained by the Crosby or the Rubin tubes, enzymatic measurements were performed simultaneously by the Dahlqvist micromethod, on −20°C refrigerated samples

Normal lactase activity is found in all cases demonstrating good conservation of the mucosa A significant decrease of about 50% in the sucrase activity and of about 40% in the total maltase activity in the test group in shown as compared with that in the reference group ($p < 0.01$) The activity of maltases 1 and 2 being unchanged the variation of the total maltase activity concerns only the thermolabile fraction

These results corroborate those previously obtained in rat and man They confirm that the sucrase activity depends on the quantity of saccharose contributed by the diet The experimental procedure, however, does not enable us to identify which sugar induces these *a* glucosidase activities

N G Asp & A Dahlqvist (Lund) *Multiplicity of intestinal β galactosidases Contribution of each enzyme to the total lactase activity in normal and lactose intolerant patients*

Three different β galactosidases have been demonstrated in the human small intestinal mucosa (1 2 3 4) The first enzyme the lactase is a brush border enzyme with optimum pH 5 5–6 Lactose is the preferential substrate and 0 2 mM *p* chloromercuribenzoate (CMB) does not inhibit this enzyme The second enzyme, the

acid β galactosidase is probably lysosomal and has optimum pH 4-4 5 It has the broadest substrate specificity of the three enzymes hydrolysing a number of synthetic β galactosides as well as lactose and is completely inhibited by 0 1 mM CMB The third enzyme the hetero-β galactosidase has optimum pH 5 5-6 The enzyme hydrolyses synthetic β galactosides but no lactose and is completely inhibited by 0 1 mM CMB

The differences between the enzymes regarding pH optimum substrate specificity and sensitivity to CMB as inhibitor made it possible to work out specific methods that could be used for assay in tissue homogenates without separation of the enzymes The acid β galactosidase could be estimated with 2 naphthyl β galactoside as substrate since this substrate was not hydrolysed by the other two enzymes The lactase could be assayed with lactose as substrate in the presence of 0 2 mM CMB as an inhibitor of the acid β galactosidase The hetero-β galactosidase was assayed with p nitrophenyl β galactoside as substrate The CMB inhibited activity was estimated at pH 6 The contribution of the acid β galactosidase was then calculated from the 2 naphthyl β galactosidase activity and the difference represented the hetero-β galactosidase activity

In biopsy specimens from 17 lactose intolerant Finlanders (5) the mean activity of brush border lactase was 3 7 U/g protein The activities of the other two β galactosidases did not differ significantly from those of controls with normal lactase activity In a group of 18 Zambians (6) the mean activity of brush border lactase was 1 7 U/g protein The activity of acid β galactosidase was higher than in the lactase deficient Finlanders while the level of hetero-β galactosidase was similar

Lactase activity is generally assayed at pH 6 where the brush border lactase has maximal activity The acid β galactosidase will then usually contribute with about 1 unit of lactase activity g protein In lactose tolerant patients therefore the acid β galactosidase will be responsible only for 2-10 % of the total lactase In lactose intolerant patients on the other hand up to 90 % of the total lactase activity at pH 6 may be due to the acid enzyme All the lactose intolerant patients studied so far have been adults Children with congenital lactase deficiency may have even lower or possibly zero levels of brush border lactase

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K. Laumala T Sahi M Isokoski J Jussila & T Niemi (Helsinki) *Lactose malabsorption in school-children*

The prevalence of lactose malabsorption (LM) in the adult population (21-65 years old) of the Finnish rural commune of Pornainen was 15 % and independent of age (Jussila J Isokoski M & Laumala K *Scand J Gastroent 5* 49 1970)

The present study concerns the school-children of the same community (7-15 years old) By random sampling 162 children out of 380 were selected Eighty per cent 130 children consented to participate in the examination There was no significant difference between the participants and non participants as regards age or sex distribution or regarding abdominal complaints as ascertained by a questionnaire

An oral lactose tolerance test (1 g per kg body weight with a maximal dose of 50 g) was performed A rise in blood glucose concentration of at least 25 mg/100 ml was considered a sign of normal lactose absorption If the rise was 20-24 mg/100 ml the test was repeated with a double dose and a rise of 20 mg/100 ml or more was taken as normal. On every child with a rise of 15-19 mg/100 ml

a small intestinal biopsy was made and mucosal maltase, sucrase and lactase activities were determined Thus the LM was based on the following criteria a maximal rise in the lactose tolerance test of less than 15 mg/100 ml, or mucosal lactase activity of less than 15 U/g protein

LM was found in 11 subjects, the prevalence being 8 5 %, and there was no significant difference in prevalence between boys and girls In the two age groups, 7–11 and 12–15 years, the prevalence was 6 and 12 %, respectively, but the difference was not statistically significant There was no difference in the occurrence of abdominal complaints, either in general or a day after the examination, between those with LM and those with normal absorption Nor was there any difference in the height or weight distributions

When the prevalence of LM, now observed in the two age groups of school children (6 and 12 % respectively), is compared with that found in the young adults (23 %) of the same community it seems likely that LM begins to appear even before the age of 7 years but that it most commonly develops between 10 and 20 years

S Auricchio M Pierro G Andria & G De Ritis (Naples) *Arylamidase activities of brush border membrane of rat intestine*

β-Naphthylamide of L-leucine L phenylalanine L-lysin and also α and γ glutamyl β naphthyl amide have been used as substrates for the assay of intestinal peptidases Crude and purified brush border and brush border membrane were prepared from rat intestine

The pH activity curves are different for the hydrolysis of the different substrates in the membrane preparation all arylamidase activities are ion dependent with the exception of that hydrolysing γ glutamyl-β naphthylamide when the activating effect of Mn^{++} Co^{++} and Zn^{++} was evident only after prolonged exposure of the membrane at 37°C to EDTA Studies on intracellular distribution demonstrate that these enzymatic activities of the brush border are due to intrinsic enzyme(s) of the membrane, and these probably play a role in terminal digestion of proteins

Methods for the assay of these membran enzymatic activities in total homogenate of intestinal biopsies could be useful in understanding the hypothetical role of intestinal peptidase deficiency in the pathogenesis of diarrhoea

R Gruttner & Th Lucking (Hamburg) *Chronic diarrhoea following partial resection of the small bowel*

On December 17th, 1967, surgical intervention was necessary on a normally developed girl, born on July 9th, 1965 due to ileus, caused by strangulation of a diverticulum of Meckel The terminal ileum the caecum and the appendix, 20 cm in length altogether, had to be resected Following the operation the girl immediately developed severe diarrhoea and no longer gained in height or weight. Coeliac disease was suggested as the introduction of a gluten free diet in April 1968 was followed by marked improvement in the diarrhoea condition At this time antibodies against gluten could be demonstrated in the blood A detailed examination at the end of 1969 in the Children s Hospital of the University of Hamburg did not point to any of the chronic intestinal malabsorption syndromes known at the present time All resorption tests performed were normal Steatorrhea could not be demonstrated Oral loading with gluten was followed by diarrhoea apart from intolerance to gluten no other cause for this could be found Diarrhoea continued for several weeks during which steatorrhea could once more not be demonstrated Oral biopsies following the oral gluten loading did not show pathognomonic changes of the intestinal mucosa resembling those of gluten intolerance Two months later oral loading with milk protein was performed Abdominal pain and partly mushy stools occurred but again steatorrhea could not be seen The renewed oral biopsy showed partial and subtotal

villous atrophy There were regions with almost total loss of villi close to regions with very small villi the tops of which were covered with irregular epithelial cells The clubbed thickenings of these villi showed lymphangiectatic vessels Following a gluten and milk free diet marked improvement of diarrhoea occurred

Discussing the mechanism of the pathogenesis an inborn or constitutional disease can be excluded for there was no sign of food intolerance prior to surgery This case reminds us of severe intestinal infections in the neonatal and in early infancy In these infections exogenous mechanisms may also cause coeliac disease like syndromes This may be due to unspecific food intolerance It is impossible to determine whether the pathogenesis of this disease is influenced by bacterial migration from the colon to the small intestine or by disturbances of the reabsorption of bile salts

J Jodl Z. Lojda N Simankova & P Fric (Prague) *Involvement of the small intestine in cystic fibrosis*

Jejunal biopsies of 18 children with cystic fibrosis were investigated histologically histochemically and biochemically Histologically the mucosal pattern can be divided into 4 groups In the 1st group (9 patients) no histological histochemical and biochemical abnormalities were found In the second group (3 patients) the mucosal pattern was slightly irregular with some deeper crypts and lower villi—the enterocytes displayed an enhanced quantity of RNA but their microvillous zone was morphologically and cytochemically normal In the 3rd group (3 patients) the mucosal pattern was also normal but the number of goblet cells was increased The crypts were enlarged and hypersecretion of a viscous mucous which adhered also to the villi was observed. In the 4th group (3 cases) a typical pattern of coeliac sprue was found Total or subtotal atrophy of the villi deeper crypts defective microvillous zone of enterocytes with lower enzyme activities (disaccharidases alkaline phosphatase, aminopeptidase), enhanced quantity of cytoplasmic RNA lower activities of non specific esterase glucose 6-phosphatase and lower activities of lysosomal enzymes In the propria cellular infiltration consisting of activated macrophages plasmocytes lymphocytes and eosinophils was found Typical changes were found also in zymograms shift of LDH isoenzymes in form of "H subunits and a deficiency of cathodic fractions of AF and aminopeptidase An improvement after a gluten free diet was observed

It is concluded that malabsorption in cystic fibrosis need to be only of pancreatogenic origin *Altered intestinal mucosa may participate* as well In some cases even a concomitant coeliac sprue can occur and this must be taken into account in the treatment of the patients

Helga Stolley & W Droese (Dortmund) *Lactic acid in milk formula the influence on absorption of nutrients and the influence on the metabolism in young babies*

In the course of long term balance studies with healthy babies during the first 3 months of life we added 0 35 to 0 5 g lactic acid to 100 g milk formula for ten-day periods Two different formulae were given. The protein poor formula consisted of one or two parts of cows milk diluted with one part of water The protein rich formula consisted of four parts of milk and one part of water

After addition of lactic acid to the milk formula excretion of nitrogen lipids potassium sodium and calcium increased significantly in the stools The acidity of urine increased to pH 4 5 Organic acids were excreted to higher extent. Ammonia and titrable acidity were increased during the lactic acid period by 100 % and more Calcium and magnesium urinary excretion were increased 30 % of the infants under study developed a metabolic acidosis including distinct clinical signs (paleness vomiting and retarded weight gain)

According to our findings the influence of lactic acid on the utilization and on the metabolism is greater the higher the protein con

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B Hadorn (Bern) Ch. Sumida M J Tarlow L Robinson & T T White (Seattle) *The activation of human pancreatic proteinases Studies on human pancreatic juice and duodenal juice of four children with intestinal enterokinase deficiency*

The activation of pancreatic proteinases in man has been studied using purified human enterokinase for trypsinogen activation and human pancreatic juice as the source of zymogens The same process has been studied in duodenal juice from patients with enterokinase deficiency as it contains pancreatic zymogens instead of active proteinases

In human pancreatic juice two molecular species of trypsinogen are present as has been shown previously by Figarella (1) and Keller (2) The two forms of trypsin found in human duodenal juice (3) appear to be derived from these two zymogens rather than to be activation products of the same zymogen The two trypsinogens have isoelectric points of approximately 4 7 and 7 0 as judged from their behaviour in the electrofocusing apparatus Both trypsinogens are activated by enterokinase The two trypsinogens appear to occur with different frequencies in the population

Duodenal juice of patients with intestinal enterokinase deficiency offers the unique possibility of studying the activation process under "physiological conditions Activation studies were made with duodenal juice from 4 such patients It is confirmed that enterokinase deficiency is the only cause of non activation of pancreatic zymogens Autoactivation of trypsinogen in the patients juice is prevented by a trypsin inhibitor which is probably identical with the trypsin inhibitor described by Keller in normal pancreatic juice

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Ch. Nordstrom & A Dahlqvist (Lund) *The cellular and subcellular localization of enterokinase*

Enterokinase (enteropeptidase, E C 3 4 4 8) which activates pancreatic trypsinogen in the small intestine is usually assumed to be secreted from the intestinal mucosa with the intestinal juice When we performed a study of the localization of enterokinase in the intestinal wall in order to define the site of its origin we found a localization which clearly indicates that enterokinase is not secreted from the deep layers of the mucosa but is located on the surface of the mucosal absorptive cells

The study was performed in rats When a piece of rat small intestine was cut out and rinsed about 90 % of he total trypsin activity but less than 5% of the enterokinase activity was removed When pieces of the duodenal wall were cut horizontally in a cryostat (1 2 3 4) in order to obtain sections from different levels in the villi and crypts respectively the major part of the enterokinase activity was found in the tips of the villi None of a series of pieces studied from different parts of the duodenum revealed any demonstrable enterokinase activity in the crypts or in the Brunner glands The distribution profile along the villi for alkaline phosphatase activity was as apical as that of enterokinase activity while the sucrase activity was more basically located in the villi and decreased in the tips

We also isolated brush borders from the duodenal epithelial cells using essentially the method described by Miller & Crane (5) The specific enterokinase activity (units per mg of protein) was more than 10 times higher in the isolated brush border fractions than in the original homogenates

In spite of the fact that enterokinase thus like the disaccharidases is a brush border enzyme proportionally much greater amounts of enterokinase than disaccharidases were found in human duodenal juice after a test meal indicating that the functional and anatomical localization of enterokinase may not be identical If the enterokinase activity is presented

tent of the milk formula and the younger the infant

The prevalence of metabolic acidosis with the high protein formula could be explained by the fact that increased protein intake is equivalent to increased intake of organic acids and consequently increased metabolic rate of organic acids

A second programme was started in order to discover the reasons for the poor tolerance to lactic acid in young babies The lactic acid used constitutes 80% L (+) and 20% D (−) lactic acid We examined the excretion of a number of organic acids in urine under non-acidified and under acidified cow s milk mixtures From day 6 after birth till the end of the third month lactic acid constitutes less than 1% of the total organic acids After addition of 0 35 g of lactic acid per 100 g milk formula there was an increased excretion of organic acids in urine Lactic acids increased from less than 1% to 10% of the total organic acids There was a decrease of citric acid during the lactic acid feeding period The excretion of lactic acid in urine is mainly D (−) lactic acid (80%)

During our study we observed one infant with severe lactic-acid intolerance

The results led to the following conclusions

1 lactic acid is metabolized more slowly the younger the infant
2 D lactic acid is utilized by the infant to a much lower degree than L lactic acid
3 D lactic acid is supposedly responsible for the detrimental effects of the addition of lactic acid to cow s milk mixtures
4 Excretion of citric acid in urine was found to be a sensitive indicator for the acid base metabolism of the infant

U Spahn Ursula Eltz & W Plenert (Jena)
Serum lipid pattern during infectious hepatitis in children Influence of dietary management

The acute phase of infectious hepatitis was associated with remarkable changes in serum lipids even in patients with less severe course of this disease Since nearly all lipid classes were affected in a different manner, the observed biochemical abnormalities are difficult to interpret

The serum free fatty acids (FFA) were found to be significantly increased initially in most of the cases In agreement with the clinical aspects and serum transaminases they returned to normal values in the second and third week of the treatment, respectively No differences could be established concerning this parameter between children treated with a usual liver diet" and those, who were on a normal diet containing butter fat even in the beginning treatment Esterified cholesterol of all fractions showed a significant fall, while free cholesterol was found to be markedly increased

Thus the ratio cholesterol ester total cholesterol was considerably diminished to an average of 30 compared to 65 in normal children Within 3 weeks of hospitalization this ratio returned to normal, while the relative distribution of cholesterol esters remained abnormal, since there was now a decreased percentage of linoleate in all children, who had been on a liver diet" In only 5 of the patients fed with a normal diet had the percentage of cholesterol esters become normal The initial drop in esterified cholesterol may be due to a reduced rate of cholesterol esterification while the decrease in cholesterol linoleate may be due to an insufficient fat supply In contrast to these changes in cholesterol ester concentration total esterified fatty acids in serum tended to be elevated They seemed however to be largely unaffected by dietary management Serum phospholipids were affected in a characteristic manner for acute hepatitis

There were reasonably close correlations between total cholesterol free cholesterol, total lipid phosphorus and lecithine Sphyngomyelin and lysolecithin rose somewhat and lecithin rose markedly The abnormalities in serum phospholipids are suggested to be mainly due to the metabolic disorders in the diseased liver To some extent they may be caused by a transitory bile retention

probably represent the maximal evacuation power of the infant stomach at this age

It is concluded that within a few minutes after feeding the young infant glucose or milk meals there are mechanisms operating which slow down the gastric emptying rate so that the stomach empties 2 to 3 times below its maximal capacity As the influence of the vol ume varies for different kinds of meal gastric emptying rate seems to be determined by the interaction between volume dependent gastric propulsive activity and inhibitory mechanisms influenced by the composition of the meal

F Drillet F Rey & J Rey (Paris) *Influence of the rate of perfusion on the in vivo kinetics of glucose and amino acid absorption*

A technique of intestinal perfusion with a double lumen tube was applied to 24 children ranging from 6 months to 5 years age Glucose leucine and lysine solutions (rendered isotonic by addition of NaCl) were infused in the first jejunal loop Intestinal absorption was calcu lated on 20 cm

Analysis of the influence of the rate of in fusion (1 2 – 2 8 – 3 6 and 4 5 ml/min) has demonstrated that intestinal absorption increases in proportion to the flow and that this is not related to any modification in the concentration of the substrate along the segment examined. A study on the kinetics of absorption at two different rates (3 8 and 7 5 ml/min) has shown moreover that absorption is not proportional to the substrate concentration but depends on the number of molecules perfused per unit of time the maximum absorption capacity is identical for the two rates eliminating the hypothesis that this effect may be related to a variation in the surface of the studied loop

The mechanism of intestinal absorption of glucose and amino acids in an open system is therefore identical to that of the kidney (Tm limited) The concept of Km has in this case no significance the system being defined by its maximum capacity and its half saturation loading.

H Loeb G Vandevelde S Godart M Gold stein A Piepsz & J Otten (Brussels) *Protein losing enteropathy with anomalies of intestinal lymphatics diagnosis and treatment*

The case report concerns a girl aged 12 years presenting progressive generalized oedema and vague intermittent abdominal pain Laboratory data revealed a hypoproteinemia without al-buminuria as well as a lymphocytopenia Nu tritional deficiency kidney and liver diseases were ruled out Intestinal protein loss was dem-onstrated with 131 I labeled albumin and albu-min 51 Cr Intestinal transit and jejunal biopsy were normal A lymphography disclosed gen-eralized lymphangiectasia with reflux in dilated mesentric and coeliac vessels The diagnosis was confirmed at laparotomy and the dilated prepancreatic vessels were ligated and cut Two months afterwards serum proteins became nor-mal and isotopic tests confirmed the disappear ance of the intestinal protein loss However 5 months after the operation a chylous ascitis developed progressively serum proteins re mained normal A peritoneo-venous anastomo-sis was carried out which proved unsuccessful after 2 weeks Thereafter ganglio-venous ana stomoses were undertaken allowing a satis factory lymphatic drainage ascitis disappeared gradually

L L Kulczycki (Washington) *Malabsorption with vitamin A deficiency in a college girl treated for cystic fibrosis*

White female 19 years old wt. 46 kg het 155 cm (5–10) a known case of cystic fibrosis with excellent progress until recently was hospitalized with acute bronchial pneu monia.

Following admission these additional mani festations were revealed (*a*) night blindness and diminished day vision deteriorating signifi-cantly within the past 3 months (*b*) anosmia (*c*) amenorrhoea for the past 8 months (*d*) absent DTR and questionable ataxic gait (*e*) subclinical malabsorption A suggestion of brain

to the intestinal lumen by cell desquamation or by some hitherto unknown release mechanism acting on intact cells in the villi remains to be explained

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G Zoppi, G Andreotti, F Pajno-Ferrara & D Gaburro (Ferrara) *Pancreozymin–secretin test in newborns*

Pancreatic response to pancreozymin and secretin stimulations has been studied in 24 newborns 16 were premature with a gestational age between 32 and 34 weeks and did not present any evidence for respiratory or neurological illness Their weight at birth was between 2 and 2 4 kg 8 were full term healthy babies with a weight at birth between 3 6 and 4 kg

Duodenal contents were collected through a newly manufactured double balloon triple lumen plastic PVC tubing having an external diameter of 3 5 mm

The patients all receiving the same diet were tested three times, i e at birth before first feeding during second day of life and at end of first week In premature babies an additional test was performed at 1 month of age

Total output of fluid volume and pH were measured immediately after completion of the test, and trypsin lipase and *a* amylase activities were then assayed and calculated as output/kg body weight/50 min after hormonal stimulations

The measured enzyme activities present at birth in both premature and full term babies seem to diminish during first day of life and return to starting values at one week

At birth the enzyme activities found in premature babies are lower than in full term babies, and become higher 1 week later The possible role of various adaptation mechanisms is discussed in order to explain the observed patterns of pancreatic response to pancreozymin and secretin stimulations

B Cavell (Lund) *Gastric emptying in infants*

Gastric emptying patterns were studied in infants aged 2 to 12 weeks after the administration of a meal of human milk After giving the milk via an infant feeding tube, reiterated intragastric volume determinations were performed at regular intervals using a marker (polyethylene glycol) Most of the infants showed an initial, rapid emptying phase where the stomach emptied 1 to 3 ml of its contents per minute This was followed by a slower phase with a more or less constant emptying rate about 0 3 to 0 7 ml/min until the stomach was empty The basic emptying pattern could best be described by an exponential function with 1 to 3% of the gastric contents emptying per minute

The effect of varying the volume and the composition of the test meal on the emptying rate during the first ten minutes was examined in 6 infants Each infant was given three different volumes of 200 milliosmolar saline, 5% glucose and 10% glucose, respectively, so that the influence of the quantity and the quality of the meal could be evaluated simultaneously in the same infant The emptying rate proved to be dependent of the volume of the meal, i e the larger the volume the faster the emptying of the stomach This was most marked for the saline meals where a 10 ml increase in the volume raised the emptying rate by an average of 0 63 ml/min The corresponding figures for 5% and 10% glucose were 0 32 and 0 30, respectively, and, when calculated for the milk meals as a comparison, 0 16 ml/min For large meals of saline, emptying rates of about 10 to 12 ml/min were recorded Such high rates

to severe malnutrition after being fed cows milk. In three of these children absorption studies were performed Steatorrhea was present whereas lactic acid excretion was normal Jejunal biopsies were in all 4 cases completely or almost completely flat

In one case withdrawal of cows milk and its replacement by soyabean milk was followed by clinical improvement. At 5 months a repeat jejunal biopsy showed normal villous morphology

The second case was given human milk medium chain triglycerides fructose and pancreatic enzymes The clinical picture improved very slowly There was no immunological defect. At 12 months weight corresponded to the tenth percentile for age but height was below this standard At this age the villous morphology was almost normal

The third case had received several antiseptic drugs before admission. Septicemia due to colibacillus and intestinal infection due to O55B5 Colibacillus were demonstrated Diarrhoea could not be controlled by discontinuing oral feeding The patient died after surgical intervention of acute intestinal obstruction

In the fourth case an intestinal infection by Colibacillus was noted but diarrheoa preceded infection and persisted after its cure With drawal of cows milk and its replacement by soyabean milk medium chain triglycerides and fructose coincided with clinical improvement and moderate weight gain

S Nordio I Antener M Kaser & R. Gatti (Genoa) *Aminoacids of faecal ultrafiltrate in different pathological conditions*

Ultrafiltrates of 24-hours faeces from a few control subjects and some cases with different diseases (common rickets vitamin D resistant rickets pseudodeficiency vit D-resistant rickets Hartnup syndrome cystinuria Lowe syndrome maple syrup disease etc) were studied with the Stein Moor method

N Ansaldi & A. Musso (Torino) *Gastric juice immunoglobulin values in the first two years of life*

Gastric juice immunoglobulin evaluation in healthy children in the first two years of life showed a prevalence of IgM

This finding conflicts both with the literature data reported for duodenal and intestinal secretions in the adult and with immunological pictures observed in older controls in the present study

B J Schmidt J Wehba N Carvalho, J Pinus & A Carvalho (Sao Paolo) *Radioactive (R B I[131]) rose Bengal excretion test in the obstructive jaundices of the infants*

Urine and stools were collected strictly separated for a period of 72 hours divided in 3 periods of 24 hours collection. Six infants were used for control being 5 neonates 3 to 4 days old and one 65 days old The patient group constituted 46 patients 11 cases of biliary atresia 27 cases of temporary obstructive jaundice 1 case of inspissated bile syndrome 1 case of biliary atresia with situs inversus totalis and supra numerary spleens

The very early age and jaundice do not represent any contraindication for the use of the R B I[131] excretion test. Urinary excretion was of no importance The results obtained indicate the accuracy of the faecal R.B I[131] uptake determinations as a reliable test in the differential diagnosis of obstructive jaundice of infants

CROHNS DISEASE AND ITS RELATION TO ULCERATIVE COLITIS

N O Berg (Lund) *Morphologic aspects*

E Boijsen (Lund) *Angiographic characteristics*

R. Lagercrantz (Stockholm) *Immunological aspects*

Most patients with ulcerative colitis have elevated humoral antibody titres to antigen(s) from

tumor was ruled out by prompt neurological consult

Ophthalmological consult confirmed visual acuity right 20/80 and left 20/50, xerosis of cornea and conjunctiva bilaterally, and elevated dark adaptation with reduced rod and cone function to 30% measured by electroretinogram (ERG) The other laboratory data revealed *pneumonic infiltrations* by X-ray Liver function tests-borderline, with total bilirubin 2, indirect 1 75 Absorption studies, D-xylose, 5 hours urine excretion 15% (borderline) Vitamin A fasting level 19 µg/100 ml, after loading with Vitamin A 66 µg/100 ml (normal 150 µg/100 ml) Loss of 33% of fat intake and loss of 22% of protein Serum cholesterol level 166 mg/100 ml and plasma triglyceride 120 mg/100 ml, both normal

In addition to her usual diet, with pancreatic enzymes and multivitamin supplements, the patient was put on 100 000 units oral Vitamin A daily, and after 1 month showed marked clinical and symptomatic improvement Repeat ophthalmologic examination revealed visual acuity right eye 20/30, left 20/30, conjunctiva moist with good lustre There was no longer any epithelial oedema or corneal staining Blind spot was still enlarged Repeat ERG showed rod function still only 30% of normal, but *cone function was the lower limit of normal* Schirmer test revealed test strip moistened in three minutes

This patient exhibited all features of Vitamin A deficiency and cystic fibrosis

B Vitek O Saxl & O Teyschl Jr (Brno) *Investigation of liver metabolism in mongolism by means of catheterization*

To study the metabolism of carbohydrates and lipids in Down s Syndrome we catheterized mongoloid children aged 2–12 years (4 trisomies 2 translocations and 1 mosaicism) 6 were given glucagon i m 1 epinephrine Blood samples from the V cava sup and from the V hep were analysed for glucose, lactate, pyruvate and non esterified fatty acids levels After stimulation, hyperglycaemia was found in the V hep and in the V cava sup, the former being more marked The highest elevation occurred after 15 min, the peak level being reached between 30–60 min After 90 min in 2 subjects only (1 translocation and 1 mosaicism) the glucose level had returned to normal in both blood samples In the other subjects the glycaemia was still highly increased

Thus the glucogenolysis after glucagon or epinephrine did not differ from the normal response, indicating that the glycogen content of the liver is normal in spite of the fatty infiltration

While investigating the levels of lactate and pyruvate 5 subjects showed after 12 hours fasting elevated levels of lactate in both samples, the blood levels in the V hep being thrice the normal amount. In all children glucagon or epinephrine lowered lactate in the V hep to subnormal levels within a few minutes, the systemic levels being only slightly affected

The pyruvate levels were investigated in 4 children Glucagon or epinephrine caused a decrease during the first 15 min The amount of non esterified fatty acids was also elevated in both blood samples, more in the V cava sup After glucagon or epinephrine the reaction of the patients was the same as that of healthy *children*

The results confirm the existence of an abnormal regulation of carbohydrate metabolism but we did not find any changes in lipolytic response in our patients For the time being we cannot explain the occurrence of the high fasting hyperlacticemia As it prevails in the hepatic vein insufficient utilization and metabolism in the liver is probable

H Loeb M Vainsel S Cadranel, A Dachy, M Diedrickx & R Wolter (Brussels) *Malabsorption and villous atrophy in four infants relationship to cow s milk intolerance*

Four infants exhibited in their early life diarrhoea and, in one case also vomiting leading

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After stimulation, hyperglycaemia was found in the V. hep. and in the V. cava sup., the former being more marked. The highest elevation occurred after 15 min, the peak level being reached between 30–60 min. After 90 min in 2 subjects only (1 translocation and 1 mosaicism) the glucose level had returned to normal in both blood samples. In the other subjects the glycaemia was still highly increased.

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may be an extremely sensitive indicator of terminal ileal disease and the ratio is considered to be of great value as a diagnostic and consequently a prognostic guide in patients with inflammatory bowel disease

E Hess Thaysen (Aalborg) *Long term prognosis in Crohn s disease*

The epidemiology and course of Crohn s disease (C d.) is difficult to determine since the diagnostic criteria and statistical methods used vary from series to series

The disease may manifest itself at any age and differences in sex incidence are small Clinical onset before the age of 10 is rare (2-3) In approximately 80 of the cases C d develops in the second to fourth decade of life During early youth the disease tends to run a more fulminant course

In about 50 of the cases the inflammatory process is limited to the small intestine in 10 % to the colon and in 40 it involves both organs

C d is a capricious disease that is usually slowly progressive Many patients may however experience long periods of clinical remission In most cases complications such as stenosis the development of fistulae or abscess formation will eventually necessitate surgery The frequency of recurrence after apparently curative major surgery increases with the period of observation and is usually quoted to be in the range of 50 Those patients requiring a second or third resection have a 50 % chance of reasonable recovery after each operation

About 25 cases of malignant tumours in the small intestine have been described in association with C d This is not sufficient for statistical evaluation but nevertheless it should lead to some reflections on a possible connection between the two diseases In C d of the colon the incidence of carcinoma is 0 5 % as opposed to 9 0 % in prolonged ulcerative colitis (over all incidence is 0 06 %) Thus in both diseases the incidence of malignancy is significantly increased

In several series usually covering a span of three to four decades, the mortality in C d is estimated to be about 10 % A specific mortality rate cannot be assessed from these surveys In a recent paper (*Lancet I* 1135 1970) the sex age and years at risk for each patient has been considered in order to evaluate the death rate The mortality registered was more than twice the number expected for a normal population of similar age and sex distribution The excess number of deaths was significant for both sexes and for those who had developed symptoms before the age of forty

G W Meeuwisse & B Hansing (Lund) *Medical versus operative treatment*

A retrospective study has been carried out on the cases of regional enteritis (Crohn s disease) (RE) and ulcerative colitis (UC) admitted for the first time before the age of 15 to the Lund hospital during two consecutive 7 year periods 1956-62 and 1963-69 It was attempted to make exact diagnoses on the available data In the first period there were 2 sure cases of RE (2 girls) 1 doubtful case 2 cases of either RE or UC and 2 sure cases of UC (2 boys) In the last 7 year period the incidence of both diseases was higher 14 cases of RE (10 boys 4 girls) and 17 cases of UC (3 boys 14 girls) In this period the sex distribution corresponds to most reports in the literature During the first 7 year period RE was not treated with long term administration of drugs The RE patients were treated surgically Several relapses requiring new resections occurred and as malnutrition developed the results were disappointing.

Since 1963 RE patients have been treated with Salazopyrin except in one case a girl who due to acute initial illness was taken over to the surgical department resected and considered cured The other patients have hitherto been well or much improved Some of them have been given small doses of steroids as well as Salazopyrine during limited periods In some illustrative cases discontinuation of the Salazo-

colon These autoantibodies crossreact with antigen(s) from Escherichia coli Patients leukocytes are cytotoxic in vitro to colon mucosa cells Enteric antigen(s) inhibit in vitro migration of patients leukocytes

Antibodies against colon are also elevated in most patients with Crohn's disease These antibodies probably have different specificity than those found in patients with ulcerative colitis In vitro migration inhibition tests are reported negative in Crohn s disease According to some investigators patients with this disease also have impaired delayed hypersensitivity to tuberculin and Kveim s antigen

Both diseases respond fairly well to Salazopyrine, corticosteroids and immunosuppressives but probably require radical surgery for definite cure

The very marked difference in the incidence of malignancy in these two diseases may reflect different immunological mechanisms

E Hess-Thaysen (Aalborg) *Some reflections on the diagnosis of Crohn s disease*

Diagnostic uncertainty is in particular related to the problem of differentiating Crohn s disease of the colon from ulcerative colitis None of the features frequently referred to as more or less characteristic are in themselves pathognomonic and, in spite of firm clinical criteria correspondance with pathological features is rather poor Consequently it has been proposed that in the clinical treatment of such patients a determination of the site and extent of the disease should be considered more valuable as a prognostic guide than the clinical label attached to it In this context the demonstration of possible terminal ileal disease is of particular interest

Small intestinal radiography and selective angiography are often most helpful but these examinations may occasionally fail to disclose definite diagnostic features in this part of the gut In such cases more precise information may be derived from a detailed evaluation of the different functions of the terminal ileum

As is well known a pathological Schilling test and perhaps a low concentration of serum cholesterol may be compatible with a terminal ileopathy This may also apply to a normal D-xylose test in the presence of steatorrhoea. During recent years the interest has been further focussed on the disturbed enterohepatic circulation of bile salts in disease of the terminal ileum Recently, evidence of this type of disturbance has been found in the form of a raised glycine/taurine conjugation ratio of bile salts in aspirates from the duodenum

A Bruusgaard (Aalborg) *Localization of inflammatory bowel disease as a prognostic guide*

The evaluation of the prognosis in Crohn s disease is not an easy matter, chiefly due to the diagnostic difficulties especially concerning the differentiation of Crohn s disease of the colon from ulcerative colitis

According to Glotzer et al (*New Engl J Med* *282* 582 1970) a determination of the site and extent of the inflammatory bowel disease seems to be the most valuable prognostic guide

With regard to the site the main difficulty, but at the same time the most important factor, is usually to demonstrate the presence of terminal ileal involvement, since the conventional methods may fail

In terminal ileopathy the enterohepatic circulation of bile acids is disturbed, resulting i a in an increased ratio of glycine to taurine conjugated bile acids (G/T ratio) in the bile

A report is given of two patients with diarrhoea and slight steatorrhoea due to inflammatory bowel disease localized in the terminal ileum Their G/T ratio was demonstrated to be increased even before the conventional methods showed evidence of terminal ileopathy The diagnosis of Crohn s disease was later confirmed by operative and microscopic findings

Bile acid analysis was performed according to the procedure described by Bruusgaard (*Clin Chim Acta* *28* 495 1970)

It is concluded that an increased G/T ratio

may be an extremely sensitive indicator of terminal ileal disease and the ratio is considered to be of great value as a diagnostic and consequently a prognostic guide in patients with inflammatory bowel disease

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Cd is a capricious disease that is usually slowly progressive Many patients may however experience long periods of clinical remission In most cases complications such as stenosis the development of fistulae or abscess formation will eventually necessitate surgery The frequency of recurrence after apparently curative major surgery increases with the period of observation and is usually quoted to be in the range of 50 Those patients requiring a second or third resection have a 50 chance of reasonable recovery after each operation

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Since 1963 RE patients have been treated with Salazopyrin except in one case a girl who due to acute initial illness was taken over to the surgical department resected and considered cured The other patients have hitherto been well or much improved Some of them have been given small doses of steroids as well as Salazopyrine during limited periods In some illustrative cases discontinuation of the Salazo-

colon These autoantibodies crossreact with antigen(s) from Escherichia coli Patients' leukocytes are cytotoxic in vitro to colon mucosa cells Enteric antigen(s) inhibit in vitro migration of patients' leukocytes

Antibodies against colon are also elevated in most patients with Crohn's disease These antibodies probably have different specificity than those found in patients with ulcerative colitis In vitro migration inhibition tests are reported negative in Crohn s disease According to some investigators patients with this disease also have impaired delayed hypersensitivity to tuberculin and Kveim's antigen

Both diseases respond fairly well to Salazopyrine, corticosteroids and immunosuppressives but probably require radical surgery for definite cure

The very marked difference in the incidence of malignancy in these two diseases may reflect different immunological mechanisms

E Hess-Thaysen (Aalborg) *Some reflections on the diagnosis of Crohn s disease*

Diagnostic uncertainity is in particular related to the problem of differentiating Crohn s disease of the colon from ulcerative colitis None of the features frequently referred to as more or less characteristic are in themselves patho gnomonic and, in spite of firm clinical criteria correspondance with pathological features is rather poor Consequently it has been proposed that in the clinical treatment of such patients a determination of the site and extent of the disease should be considered more valuable as a prognostic guide than the clinical label at tached to it In this context the demonstration of possible terminal ileal disease is of particular interest

Small intestinal radiography and selective angiography are often most helpful, but these examinations may occasionally fail to disclose definite diagnostic features in this part of the gut In such cases more precise information may be derived from a detailed evaluation of the different functions of the terminal ileum

As is well known a pathological Schilling test and perhaps a low concentration of serum cholesterol may be compatible with a terminal ileopathy This may also apply to a normal D-xylose test in the presence of steatorrhoea. During recent years the interest has been further focussed on the disturbed enterohepatic circulation of bile salts in disease of the ter minal ileum Recently, evidence of this type of disturbance has been found in the form of a raised glycine/taurine conjugation ratio of bile salts in aspirates from the duodenum

A Bruusgaard (Aalborg) *Localization of in flammatory bowel disease as a prognostic guide*

The evaluation of the prognosis in Crohn s dis ease is not an easy matter, chiefly due to the diagnostic difficulties especially concerning the differentiation of Crohn s disease of the colon from ulcerative colitis

According to Glotzer et al (*New Engl J Med 282* 582, 1970) a determination of the site and extent of the inflammatory bowel disease seems to be the most valuable prognost ic guide

With regard to the site the main difficulty, but at the same time the most important fac tor, is usually to demonstrate the presence of terminal ileal involvement since the conven tional methods may fail

In terminal ileopathy the enterohepatic cir culation of bile acids is disturbed, resulting i a in an increased ratio of glycine to taurine conjugated bile acids (G/T ratio) in the bile

A report is given of two patients with diar rhoea and slight steatorrhoea due to inflam matory bowel disease localized in the terminal ileum Their G/T ratio was demonstrated to be increased even before the conventional meth ods showed evidence of terminal ileopathy The diagnosis of Crohn s disease was later con firmed by operative and microscopic findings

Bile acid analysis was performed according to the procedure described by Bruusgaard (*Clin Chim Acta 28* 495, 1970)

It is concluded that an increased G/T ratio

may be an extremely sensitive indicator of terminal ileal disease and the ratio is considered to be of great value as a diagnostic and consequently a prognostic guide in patients with inflammatory bowel disease

E Hess Thaysen (Aalborg) *Long term prognosis in Crohn s disease*

The epidemiology and course of Crohn s disease (C d) is difficult to determine since the diagnostic criteria and statistical methods used vary from series to series

The disease may manifest itself at any age and differences in sex incidence are small Clinical onset before the age of 10 is rare (2–3 %) In approximately 80% of the cases C d develops in the second to fourth decade of life During early youth the disease tend to run a more fulminant course

In about 50 of the cases the inflammatory process is limited to the small intestine in 10 to the colon and in 40 % it involves both organs

C d is a capricious disease that is usually slowly progressive Many patients may however experience long periods of clinical remission In most cases complications such as stenosis the development of fistulae or abscess formation will eventually necessitate surgery The frequency of recurrence after apparently curative major surgery increases with the period of observation and is usually quoted to be in the range of 50 Those patients requiring a second or third resection have a 50 chance of reasonable recovery after each operation

About 25 cases of malignant tumours in the small intestine have been described in association with C d This is not sufficient for statistical evaluation but nevertheless it should lead to some reflections on a possible connection b tween the two diseases In C d of the colon the incidence of carcinoma is 0 5% as opposed to 9 0 in prolonged ulcerative colitis (over all incidence is 0 06%) Thus in both diseases the incidence of malignancy is significantly increased

In several series usually covering a span of *three to four* decades the mortality in C d is estimated to be about 10% A specific mortality *rate* cannot be assessed from these surveys In a recent paper (*Lancet I* 1135 1970) *the sex* age and years at risk for each patient has been considered in order to evaluate the *death rate* The mortality registered was more than twice the number expected for a normal *population* of similar age and sex distribution The excess number of deaths was significant *for both sexes and for those* who had developed symptoms before the age of forty

G W Meeuwisse & B Hansing (Lund) *Medical versus operative treatment*

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Since 1963 RE patients have been treated with Salazopyrin except in one case a girl who due to acute initial illness was taken over to the surgical department resected and considered cured The other patients have hitherto been well or much improved Some of them have been given small doses of steroids as well as Salazopyrine during limited periods In some illustrative cases discontinuation of the Salazo-

pyrine treatment was followed by recurring symptoms and weight loss Reinstituted therapy resulted in remission In one case, where a drug induced exanthema developed, discontinuation has not given new symptoms although X ray visible changes persist The other cases remain on treatment except one, a girl, where Salazopyrine was discontinued by the surgeon when, due to stenosis, her left colon was resected She has not been well since operation The other cases have not yet needed surgical treatment

Considering the high recurrence rate of RE after resection and the fact that microscopic changes are detectable in the gut mucosa far away from the macroscopic lesion in many of the cases, the disease ought to be regarded as a chronic, systemic disease Long term medical treatment will be most benificial when started early in the course of the disease A localized lesion may be resected, especially if obstruction is threatening, but it seems worthwhile to continue Salazopyrine treatment in such patients as well

J T Harries & June K Lloyd (London) *Azathioprine in the treatment of Crohn's disease*

We present 2 children with severe and extensive intestinal involvement who improved markedly after treatment with azathioprine in combination with a small dose of prednisolone

Case 1 The diagnosis was made at 10 years when she presented with a short history of bloody diarrhoea, weight loss and abdominal pain Initially she responded to salazopyrine and steroids but these drugs had little effect when she subsequently relapsed a year later Symptoms persisted, her nutritional state deteriorated, severe perianal fissures developed and signs of steroid toxicity developed Investigations at 12 years showed the distal ileum transverse and descending colon to be involved haemoglobin was 8 5 g/100 ml and serum iron, folate and total protein levels were reduced, faecal fat was 9 6 g/day and E S R 27 mm in 1 hr Azathioprine (2 mg/kg/day) and prednisone (0 2 mg/kg on alternate days) resulted in a striking clinical and biochemical improvement after 8 weeks The dose of both drugs were gradually reduced without clinical deterioration, but when the prednisone was stopped after 13 months there was a prompt relapse

Case 2 A 4-year old boy whose father also had Crohn's disease presented with a short history of weight loss, diarrhoea and abdominal pain There was extensive involvement of the small intestine, haemoglobin, serum iron, folate and albumin levels were reduced, faecal fat was 5 3 g/day and E S R 52 mm in 1 hr The response to azathioprine (2 mg/kg/day) and prednisolone (0 5 mg/kg/day) was striking When the prednisolone was reduced to 0 1 mg/kg/day symptoms promptly recurred and rapidly disappeared when the dose was increased to 0 3 mg/kg/day After 14 months of combined therapy his condition remains good

K H Schafer & F Blaker (Hamburg) *Azathioprine in the treatment of ulcerative colitis*

In the conservative long term therapy of ulcerative colitis psychotherapy and Salazopyrine still hold an important place Immunosuppression is on the other hand a new and obviously very effective therapeutic means As the first remedy we have chosen Azathioprine However, Azathioprine needs some weeks until it reaches its full effectiveness Therefore at the beginning of therapy we combine the Azathioprine dose (5 mg/kg/day) with 6 methyl prednisolone (1 mg/kg/day) which is quickly effective After onset of remission (usually within four weeks) 6 methyl prednisolone is slowly removed and Azathioprine is reduced to the maintenance dose of 3 mg/kg/day This therapy has been used in our hospital since 1966 for ulcerative colitis in children and we have obtained thoroughly satisfactory results Out of 14 cases there are at the moment 12 in satisfactory remission of 2 till 32 months A 13th case unaccountably relapsed under maintenance therapy of 3 mg/kg/day Azathioprine after a

satisfactory remission of 20 months In the 14th case the Azathioprine treatment was not fully effective as a reduced dose (1 mg/kg/day) had to be given due to the side effects (cholestatic jaundice) Side effects have been observed in another case where violent vomiting occurred but neither alopecia nor decreased linear growth. In general we stopped the ther apy after 24 months if there was no relapse In conclu ion our experiences so far have been encouraging However the future will show whether our remission will result in complete cures We suspect that Azathioprine is effective through immunosuppression. Arguments in favour of this are as follows the disappearance of antinuclear antibodies (in about 20% of the cases) the disappearance of lymphocytes specifically adapted against colonic mucosa (identified by the method of Neth & Blaker Klin Wschr 48 55 1970) and the return of complement C 3 in association all with clinical improvement during Azathioprine therapy

ABSORPTION IN RELATION TO BILE SALT METABOLISM

B Borgstrom (Lund) *Present concept of fat absorption*

C Ricour & J Rey (Paris) *Kinetics of fat hydrolysis and micellar solubilisation in bile and lipase deficiencies*

A duodenojejunal perfusion was performed on 16 children with a double lumen tube An in teresterified oil mixed into a test meal was perfused as a substrate (20 mg/min/m) for 4 hours Bile and lipase concentrations and the composition of the oil and micellar phases of the intestinal content were analysed

In normal children the lipase concentration (300 U D /ml) and the molar percentage of FFA (75 of the total lipids) released by hydrolysis of the substrate are constant during the perfusion whereas the proportion of FFA and MG solubilised is modified proportion ately to the bile salt concentration during the first hour it represents about 2/3 of the total lipids, between the 100th and 200th minute it only constitutes half of the initial value

In lipase deficiencies (congenital absence of lipase cystic fibrosis) the proportion of TG and DG is increased considerably leading to a significant reduction in the percentage of lipids solubilised FFA and MG are concentrated in the micellar phase In bile salt deficiencies (complete atresia of the extra hepatic bile ducts ileal resection) the micellar phase is practically non existent a diminished rate of hydrolysis is also demonstrated the importance of the interface renewal in hydrolytic process is thus underlined

F Hanna (Columbia) *The bile acids The di gestion and absorption of different triglycerides and the effects on the absorption of calcium and magnesium*

Bile acids role in calcium absorption is multi faceted The absorption of vitamin D is en hanced by bile acids. This indirectly influences the synthesis of calcium carrier protein in the intestinal mucosa and is a delayed effect The immediate effects include solubilization of the sparingly soluble calcium salts in the diet and this action explains some of the discrepancy in experimental results depending on the solu bility of the calcium source in the diet. Micelle formation and the triglyceride saponification require the presence of bile acids in the in testines Here the role of bile acids depends on the type of triglyceride in the diet Tri glycerides of unsaturated fatty acids and tri glycerides of satured fatty acids with the poor ly absorbed palmitic acid in the β position favor increased calcium absorption and the free palmitic and to a lesser extent stearic acid carry calcium as insoluble calcium soap in the stools The mechanism of magnesium absorp tion is different and hence stool magnesium does not correlate with stool palmitic and stear ic acids On the other hand sequestration of bile acids by cholestyramine did not decrease calcium absorption in one experiment. Anionic

Table 1

Diagnosis	No	Coefficient of fat absorption	
		Range	Average
Malnutrition	10	87 3–98 2	93 3
Infectious hepatitis	7	94 5–98 4	96 9
Indian childhood cirrhosis	2		94 8
Congenital biliary atresia	1		38 9
Controls	10	96 7–98 7	97 9

detergent, sodium lauryl sulfate has an effect similar to bile acids on the sparingly soluble calcium salts When bile acids are excluded from the intestinal milieu, other factors influencing calcium absorption like lactose and amino acids particularly lysine and arginine play a more important role in enhancing calcium absorption

L S Prasad & C M Khurana (Patna, India) *Quantitative determination of faecal fat in liver disorders and malnutrition by balance studies*

The aim of this study was to determine the absorption coefficient of fat on a balanced diet containing 30 to 50 g of fat per 24 hours The method of fat estimation was that described by van de Kamer et al Besides a normal control group, we included 10 cases of malnturition and 10 cases of liver disease Two of the 10 cases of *malnutrition* (age 1–7 years) had kwashiorkor and 8 had the marasmic type of malnutrition One case of malnutrition presenting growth retardation showed on pilocarpine iontophoresis sweat sodium of 92 mEq/l but the coefficient of absorption of fat was as high as 97 3% In the liver disease group (age 2 months – 12 years) seven were in the recovery phase of infectious hepatitis showing enlargement of liver but without bile pigments or bile salts in the urine, 2 cases of Indian childhood cirrhosis and 1 case of congenital biliary atresia with dextrocardia and situs inversus The results are given in Table 1

Quantitative determination of faecal fat was also carried out in healthy dogs which were put on a diet containing 50 g of fat in the form of butter (excluding moisture) and 2 litres of skimmed milk with sugar After 3 days 24 hour samples of their stools were analysed for total fat (T), hydrolysed (H) and unhydrolysed fat (U) for 5 days by van de Kamer s method The absorption coefficient of fat was calculated from the sliding mean from 3rd to 5th day's sample of stool

After ligation of the hepatic duct and a recovery period of 5 days, the dogs were again put on a balanced diet as before and the fat excretion from 4th to 8th day was determined as mentioned above

Following this, the fat excretion was determined while the dogs remained on the same diet but with the addition of Colibil (a syrup containing choline and bile compound with sorbitol) in a dose of 2 teaspoons thrice daily The results are given in Table 2

B Strandvik & A Norman (Stockholm) *Bile acid metabolism in infants with liver disease*

The metabolism of bile acids has been studied in infants with biliary atresia (b a) neonatal hepatitis syndrome (n h s) and other diseases with cholestasis After intravenous administration of cholesterol 4 ^{14}C to infants with b a isotope was mainly excreted in urine and to a minor extent in faeces The labelled products in faeces were neutral steroids and in urine mainly cholic and chenodeoxycholic acids Cholic acid 24 ^{14}C was administered intramuscularly to infants with various liver diseases and the excretion of isotope in urine and faeces followed for 4 days The nature of the labelled products and the total excretion of cholic and

Table 2 *Experimental data in dogs*

No	Sex	Age	Pre-operative		Post-operative	Post-operative + Colibil	Remarks
1	F	1yr	T	2 77	4 35	—	Dog died
			H	1 01	2.87	—	
			U	0 65	1 26	—	
2	M	2yrs	T	0 684	1 026	0 570	
			H	0 560	0 906	0 417	
			U	0 124	0 120	0 153	
3	F	1yr	T	1 095	0 655	0 456	
			H	0 895	0 494	0 366	
			U	0 200	0 161	0 075	
4	M	4yrs	T	0 986	1 055	0 837	
			H	0 518	0 919	0 588	
			U	0.268	0 136	0 269	

chenodeoxycholic acid were determined In infants with b a. n h s or other diseases with cholestasis nearly all of the excreted isotope appeared in urine Only trace amounts of labelled unconjugated cholic acid were excreted in urine In addition to glycine and taurine conjugates other conjugates of cholic acid were excreted No 7 α dehydroxylation of cholic acid was observed All patients had a high daily urinary excretion of cholic and chenodeoxycholic acid. There were no differences in total urinary excretion of bile acids or in the metabolism of cholic acid between infants with b a n h s or other diseases with cholestasis The faecal fat excretion was increased and the absorption of vitamin A and triglycerides were impaired when the urinary bile acid excretion was increased Improvement of the fat absorption was observed when the bile acid excretion became normal

B Borgstrom A Nordén & E Ståhl (Lund) *Primary (?) bile acid deficiency in a patient with a malabsorption syndrome*

A 24 year-old woman has suffered from diarrhoea since birth At 7 years coeliac disease was suspected but the diagnosis was not confirmed In 1964 she had severe bleeding following a tooth extraction. Vitamin K deficiency but no other coagulation abnormality was found In 1967 she appeared again with bleeding symptoms She had not taken vitamin K During the last 4 years she had had increasing difficulties with her legs and could only climb a stair with the aid of her hands Her height was 152 cm and she weighed 41 kg She had absence of muscle reflexes and vibratory sense in both legs and Babinski s sign was present bilaterally suggesting combined degeneration of the cord but there was no evidence of vitamin B_{12} or folate deficiency Vitamin A treatment restored her deficient dark adaptation Plasma tocopherol was low There was no evidence of vitamin D malabsorption Daily fat excretion was 40–50 g Pancreatic lipase amylase trypsin and chymotrypsin were normal The histology and disaccharidase and dipeptidase activities of the small intestinal mucosa were normal Xylose glucose and lactose tolerance was unaffected X ray examination of the intestine showed a few wide loops of doubtful significance

Serum bilirubin alkaline phosphatase GOT and GPT were normal Serum cholesterol was 266 mg with 195 mg esterified Urinary urobilinogen was occasionally positive The bromsuphalein clearance test showed a 19 retention at 30 min and a subsequent rise to 29% at 60 min persisted for at least another hour Cholecystography was normal A liver biopsy showed only minor histological changes with some pigmentation of lipofuscin type Electron microscopy showed increased smooth

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from the loop These findings correlate with the ratio of unconjugated to conjugated bile salts found by semiquantitative thin layer chromatography in the luminal contents from each of these three areas

References

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G W Meeuwisse

surface endoplasmic reticulum Microvilli were short or absent Lipofuscin like particles were found in lysozomal structures A few bile thrombi were found in bile canaliculi

Duodenal aspiration following a test meal was done twice Thin layer chromatography and gas chromatography showed low content of bile acids On stimulation with cholecystokinin i v the same deficiency was found, the total concentration of bile acids being 8 8 μmoles/ml, all conjugated and with a glycine to taurine ratio of 0 8 By measurement in faeces and urine a half life of 5 5 days of intravenously injected sodium cholate-24-^{14}C was found, 25 % of which was eliminated via the urine Normally, only trace amounts will appear in the urine A parallel study in a patient with severe primary biliary cirrhosis showed an excretion in the urine of 15 % of the dose Thus our patient was found to have a slightly reduced elimination rate of labelled cholate and a urinary excretion exceeding the excretion seen in a patient with severe parenchymatous liver disease

The patient may suffer from a limited possibly specific defect in her bile acid metabolism Attempts to study bile acid synthesis were made by injecting labelled cholesterol intravenously, but the radioactive dose proved to have been too small The defect appears to have been present at birth Chromosomal studies have shown a normal pattern Studies of the family have included liver function and in most relatives, fine needle liver biopsies Only one sister had had signs of liver pathology with jaundice during pregnancy She had no signs of liver disease when examined Four other siblings, both parents 12 siblings of the patient's father and 5 of her mother all showed no signs of a disease resembling that found in the patient Hence, we have no evidence that the disease represents an inherited defect Substitution with bile acids has reduced stool frequency and the steatorrhoea At present the major complaint is walking difficulties Muscle histology shows changes resembling those found in rabbits on a tocopherol deficient diet We have given the patient tocopherol orally and intramuscularly for 1 ½ years with no subjective or objective signs of improvement. The plasma tocopherol level has been kept normal Recently we have started intensive treatment with vitamin B_{12} even though there have been no signs of vitamin B_{12} deficiency In addition she receives vitamin A and vitamin K

M Gracey, Valerie Burke & A Oshin (Birmingham) *Effect of deconjugated bile salts on intestinal sugar absorption*

Though abnormalities of fat and vitamin B_1 absorption are widely recognized in the 'blind loop' or contaminated small bowel syndrome, little is known of the state of carbohydrate absorption in this disorder Our clinical observation (1) that some young babies with abnormal small intestinal flora and deconjugated bile salts have impaired monosaccharide absorption, has stimulated us to investigate experimentally the effect of bile salts on intestinal sugar absorption

Using Semenza s (2) in vitro system we have found uptake of glucose and its actively transported, non metabolized analogue arbutin (*p* hydroxyphenyl-β glucoside) to be impaired in the small intestine of the normal rat in the presence of 0 5 mM sodium deoxycholate, while there is no inhibition by sodium taurocholate at a concentration of 10 mM The inhibition caused by unconjugated bile salt is reversible, and is not related to non specific tissue damage

We have further investigated this disturbance in rats with surgically constructed jejunal blind loops The absorption of glucose and arbutin, was studied in rats with jejunal blind loops 4–8 weeks after operation Uptake of these substances was studied in vitro in the small intestine proximal to the loop in the loop itself and in the small intestine distal to the loop Inhibition of sugar uptake is maximal in the blind loop and is abnormal in the entire small bowel distal to the loop and is normal only in proximal small [illegible] away

transfusion service morbus hemolyticus neonatorum, prophylactic and epidemic medical problems in blood transfusion service and consumption coagulopathies One of the sections deals with the genetic regulation of protein synthesis and gives also aspects on the antigen structure of human immunoglobulins and lipoproteins. Special articles on the polymorphism of Gc, Hp, and other serum group systems can also be found in this section

Another part contains communications in which different aspects of the Rh hemolytic disease are discussed chemical analysis of amniotic fluid intra uterine blood transfusions, and above all prophylactic treatment with anti D immune globulin. The section of coagulopathies covers the theme of intravascular clotting from both experimental and clinical point of view The clinical part contains reports dealing with etiological diagnostic and therapeutic aspects of this really important clinical problem

Bengt Low

M Adinolfi (ed) *Immunology and development* Clinics in Developmental Medicine No 34 W Heinemann Medical Books Ltd London 1969 187 pp illus 63s

The development of immunological competence in man is a very complicated process beginning early during fetal life Knowledge of the immune mechanisms in primitive species and of the phylogenetic development of the immune system is essential for the understanding of the ontogenetic development In turn, knowledge of ontogeny is very important in judging suspected states of defective immunity in infancy and childhood

This book is mainly a review of the rapidly increasing amount of knowledge in this field but it also gives information of recent scientific results some of which are not yet published elsewhere The book is primarily intended for paediatricians and immunologists but is undoubtedly of great general interest. For the practising clinician lacking a more profound knowledge of immunology some of the chapters are perhaps too theoretical and difficult to read while others are of greater practical value Owing to the collected amount of knowledge and the richness in valuable references the book is clearly of interest for the paediatrician however

The book consists of six chapters, written by different authors The first chapter by Dr D W Talmage deals with the nature of the immunological response The condensed account demands a great deal of the reader The discussion on the genetic basis of immunological diversity should be of great interest for immunologists and also for geneticians The chapter written by Adinolfi & Wood on ontogenesis of immunoglobulins and components of complement in man is clear and well written and contains a great deal of information of theoretical as well as of practical value Clem & Leslie s report on phylogeny of immunoglobulin structure and function is of great interest and seems well justified in a book like this Immunological processes in mammalian reproduction are discussed by Dr W D Billington. The confusing fact that the fetus normally avoids any homograft rejection in spite of genetic dissimilarity between mother and offspring may be explained by a masking of the histocompatibility antigens of the trophoblasts by a negatively charged mucoprotein coat. Treatment with neuraminidase seems to be able to expose the underlying cell surface antigens

The last two chapters of the book are concerned with clinical questions Dr J R. Hobbs survey of primary immune paresis deals with clinical patterns of defective immunity in childhood His classification of these conditions is very detailed but at the same time of practical value though some of the investigations recommended are only available in highly specialized centers The statement that the majority of individuals with isolated IgA deficiency have symptoms does not agree quite well with recent Scandinavian findings. Whether patients suffering from ataxia telangiectasia are susceptible to infections because of IgA deficiency or because of defective cellular immunity may perhaps also be discussed Dr R. B McConnell s chapter on the immunological relationship between mother and fetus deals mainly with immunization of the mother by passage of erythrocytes, leucocytes and platelets from the fetus The theoretical basis for prevention of maternal Rh immunization by treatment with γG anti D is discussed

The book can be highly recommended to physicians interested in paediatrics and immunology

Torsten Berg

Earl J Brewer Jr (ed) *Juvenile rheumatoid arthritis* Vol VI Major Problems in Clinical Pediatrics Saunders Philadelphia, London and Toronto 1970 231 pp illus £5 6s

Brewer is the head of the Arthritis Clinical Research Center Texas Children s Hospital of Houston Texas. His recently published book on Juvenile Rheumatoid Arthritis (JRA) is appreciated JRA is little dealt with in pediatric literature though this disease is not more rare than juvenile diabetes mellitus or nephrosis. The methods for treating JRA are still unsatisfactory and the disease is crippling to a frightening extent

Those expecting thorough discussion of immunological theories will perhaps be disappointed Instead the book gives an excellent picture of the clinical manifestations of juvenile rheumatoid arthritis in its various forms from the fulminating type called morbus Wissler by many pediatricians, to the seemingly innocent monoarticular type Differential diagnosis is well dealt with The author stresses the less known complications such as pericarditis, marked leukocytosis chronic iritis (beginning without symptoms and thus dangerous) lesions of the cervical joints with neurologic complications and local growth disturbances that may contribute to joint deformities. Radiographic changes and serology are dealt with in

BOOK REVIEWS

K Adamsons (ed) *Diagnosis and treatment of fetal disorders* Springer Verlag Berlin Heidelberg and New York 1969 304 pp illus DM 59 20

The establishment of prenatal medicine as a separate subspeciality in the 1960s has led to a productive integration of obstetrics pediatrics and basic medical disciplines This integration of disciplines has resulted in a growing concept of the fetus as a patient to whom diagnostic and therapeutic procedures could and should be offered with the same effort and intensity as with other patients However the application of advanced techniques to the study of prenatal events has resulted in a rapidly increasing amount of information which is already difficult for the individual physician to survey

This book gives a comprehensive view of the goals already achieved in this field and also of the future possibilities as to diagnosis and treatment of disorders affecting the human fetus in the gestational period and during the process of birth The book is the result of an international conference held in Puerto Rico October 1967 under the auspices of the Department of Obstetrics and Gynecology Columbia University New York and participated in by a selected group of leading authorities within this new field of medicine

Twenty two papers are presented in the book which is divided into five sections

The first section deals with the diagnosis of fetal conditions by means of morphologic and cytogenetic methods and gives a good review of the present concept of fetal deprivation and growth retardation and also of diagnostic possibilities offered by histologic metabolic enzymatic chromosomal and other determinations in placental and fetal tissues obtained by antepartum biopsy techniques

The second section concerns metabolic studies on the cellular growth patterns of the placenta under different conditions and on placenta and the feto placental unit in the synthesis and transfer of proteins amino acids and hormones Those papers are of great theoretical interest and give suggestions concerning the complex interrelationship between abnormal fetal growth and biochemical abnormalities in the placenta in terms of altered synthesis and content of proteins DNA and RNA, cell number and other factors as found in maternal diabetes maternal malnutrition and conditions of intrauterine growth failure

The third and fourth sections deal with clinically applicable new ways of assessing and monitoring the condition of the human fetus near term or in labor namely amnioscopy biochemical analysis of the amniotic fluid, acid base determinations of the fetal scalp blood and biophysical techniques for fetal electrocardiography and fetal heart rate determinations An extensive and world wide interest is currently focused on these procedures as potential means of solving the diagnostic difficulties pertaining to *fetal distress* The papers presented give as a whole a good survey of the clinical possibilities and limitations and the basic theoretical problems related to these new methods These sections should be very informative to the individual obstetrician who attempts to adopt these procedures in his own department

The fifth section entitled "prenatal treatment" comprises papers mainly concerned with intrauterine fetal transfusion in erythroblastosis fetalis a method which has not met with any enthusiasm in Scandinavia One paper however surveys the development of prevention of Rh immunization by means of passive Rh immunoglobulin a method which recently has been introduced on a broad scale in Sweden as in several other countries The section ends with an interesting paper on experimental enzyme inductions in fetal and newborn animals making it possible to interfer selectively with different metabolic reactions in order to study e g teratogenic and other effects of drugs

Due to the book s broad approach and high quality it unquestionably holds a prominent position in current literature on prenatal medicine The opinion of the referee is that it should be read by all obstetrician and pediatricians as well as by other interested in this important field of medicine The book might be considered as a textbook of prenatal medicine for medical students pertaining to the resolution on medical education adopted by the International Pediatric Association and by the General Assembly of the World Congress of Obstetrics and Gynecology in New York 1970

Lennart Jacobson

L P Hollander & M Matthes (ed) *Ergebnisse der Bluttransfusionsforschung X* Bibliotheca Haematho logica No 32 Karger Basel and New York 1969 348 pp 164 illus sFr 79

Proceedings from the 13th meeting of the Deutschen Gesellschaft fur Bluttransfusion in Graz May 1968 form the 10th volume of the *Ergebnisse der Bluttransfusionsforschung*

The proceedings comprise 41 papers most of them by invited speakers The following subjects are covered more or less in the different sections the polymorphism of serum groups automation in blood

Acta Pædiat Scand 60 385–398 1971

INFLUENCE OF ENVIRONMENTAL TEMPERATURE AND ACIDOSIS ON LIPID MOBILIZATION IN THE HUMAN INFANT DURING THE FIRST TWO HOURS AFTER BIRTH

BENGT PERSSON and RAGNAR TUNELL

From the Departments of Paediatrics Karolinska Institutet S t Gorans Sjukhus and Karolinska Sjukhuset Stockholm Sweden

Lipids are believed to be a minor source of energy for the human fetus (11) In accordance with this assumption are the recent findings of low concentrations of free fatty acids (FFA) in human fetal scalp blood (51) and the very low concentrations of FFA and glycerol in cord blood (29 38) The postnatal rises in plasma concentrations of FFA (9 12 21 22 29 38 41) and glycerol (29 38) the increases of plasma triglyceride (38) and blood ketone concentrations before the first feeding (2 25 26 38 39) and the fall in respiratory quotient (10) all indicate that the newborn infant increases lipid mobilization and oxidation of fat within the first hours after birth Several possible explanations for this increase in lipid mobilization have been suggested (9 12 21 27 38) but the most comprehensive explanation which is based on experimental evidence (13 14) is the increase in sympathetic tone that occurs after birth

It is important to note that changes in the rate of mobilization of FFA from adipose tissue are not only controlled by factors which stimulate or inhibit lipolysis but also by variations in the uptake re esterification and possibly oxidation within adipose tissue of FFA

This study was supported by grants from the Swedish Research Council (project K68 19X 1035-03 and B71 13P 3 68-01) Karolinska Institutet Reservationsanslaget and Semper Fond for naringsforskning

Recent *in vitro* studies indicate a different regulation of these processes in the newborn human adipose tissue as compared to older infants and adults (18 30 31 32 33)

The postnatal rise in plasma FFA concentrations in newborn rabbits occurs only at an environmental temperature below 33 C (47) Systematic studies on the possible influence of the thermal environment on postnatal lipid mobilization in the human newborn infant have not been reported

An antilipolytic effect of severe acidosis has recently been demonstrated (40 52) Experimentally induced hypoxia in puppies inhibited lipolysis even when the animals were subjected to cold stress (3 4) *In vitro* and *in vivo* results indicate that lactate and β hydroxybutyrate can also inhibit lipolysis (5 6 7 28)

Because of the complexity of factors affecting lipid mobilization the lack of information concerning several of these factors and the different sampling and analytical techniques interpretation of previous studies in the human infant on postnatal lipid metabolism is difficult

The aim of the present study was to investigate the possible influence on postnatal lipid mobilization in the newborn human infant of labor and delivery different environmental temperature degree of acidosis as well as the concentrations of lactate and β hydroxy

special chapters One must remember however that as a rule pathologic X ray findings appear only after the disease has lasted a few years and that rheumatoid factor is seldom present Physiologic aspects partly of a speculative nature are given disproportionately large space compared to therapeutic exercise and physical therapy Therapeutic exercise is presented only in the form of home treatment by parents and the methods are quite crude The author completely omits modern physiotherapy for rheumatoid arthritis where non weight bearing exercises especially in a pool precise traction methods articulation and isometric strength training are of great importance for the prognosis In the chapter on orthopedic treatment an attempt is made to analyze the mechanics involved in correcting knee contractures However this analysis is far too superficial The importance of resulting joint forces that cause cartilage destruction and infarctions when bracing and exercises are improperly performed is completely neglected

Drug therapy is extensively treated although chloroquine is hardly given any space in spite of its being a far better anti inflammatory drug than indomethacin Eye complications when using chloroquine are strongly over-emphasized by the author at least according to Swedish experience

Finally there is a short presentation of surgical methods both early procedures such a synovectomy and later reconstructive measures e g osteotomy

In a patient material presented by Brewer (100 patients) 33 were functionally restored 51 % developed slight disability 11° were severely crippled and 5 died This is a good result compared to other materials where severe disability is reported in 20-30% Brewer stresses the prognostic importance of early treatment at a specialist clinic with an experienced team of pediatricians rheumatologists, orthopedic surgeons physiatrists and physiotherapists Those lacking such resources should refer their patients to special clinics

Helena Svantesson

Troubles vasculaires de l enfant et de l adolescent Symposium organized by the Société Française de Phlebologie L Expansion Paris 1970 172 pp illus F 37

At least in Sweden vascular diseases in childhood appear to be sparse This impression is based on the reviewer s experience from a department of vascular surgery in which about a dozen children are examined every year as contrasted against approximately 7 000 adults

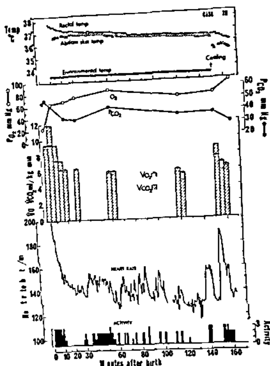
In 1967 however a questionnaire concerning the frequency of arterial and venous diseases in children was mailed to 49 experienced angiologists and pediatricians in France Belgium the Netherlands and some other Frenchspeaking countries The answers were analyzed by Dayras & Griton Paris It turned out that angiologic diseases appeared to be more common than previously assumed The majority of the physicians stated such cases to occupy between 1 and 2 of their practice the upper limit was 7 % by some dermatologists including angiomata in vascular diseases Among other interesting figures may be mentioned 110 cases of the Klippel Trenaunay syndrome post operative thrombosis of deep veins in 30 cases with appendectomy as the main pathogenetic agent and varicose veins amounting to 0 5-2 % of phlebological practices

This investigation including comments by several distinguished angiologists served as a basis for a symposium sponsored by the French Society of Phlebology in 1967 and is recently published to either with several interesting contributions to pediatric angiology May the reviewer be excused for a deep sigh is it really necessary that printed reports from symposia so very often are published years after their original presentation? In this case the publication was three years late and it cannot be helped that in some cases part of the freshness is lost

Several contributors deal with the problem of deep thrombosis in infancy There are however no hints of any diagnostic or therapeutic procedure different from those used in adults In this field one gets a remarkable demonstration of the danger of late publication since the treatment of thrombosis with streptokinase now a routine method in angiologic clinics is not even mentioned

According to my personal view the most valuable paper is that by the Dutch master of phlebology H R van der Molen on the syndrome of Klippel Trenaunay i e cutaneous hemangioma osseous hypertrophy and venous insufficiency with its usual complications all confined to one leg or one arm Van der Molen presents a broad review and gives advise on the therapy The possibility of arterio-venous anastomoses in the affected limb is clearly pointed out It should be stressed, however that several phlebologists particularly of the Scandinavian school prefer surgery rather than sclerotherapy in this disease

Knut Haeger



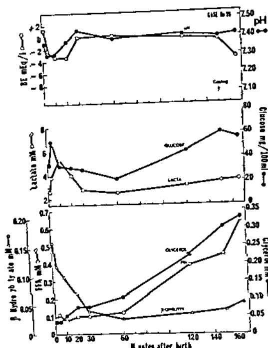


Fig. 1. Sampling times and parameters measured in a normal infant (case 28, Table 1). Note the effect of cooling at 150 min.

butyrate. A group of fullterm newborn infants were studied during the first 2 hours after birth in controlled environmental temperatures and simultaneous serial determinations of pulmonary gas exchange, heart rate, blood gases and acid base balance in these infants with special emphasis on the changes occurring during the first minutes after birth will be published separately.

MATERIAL AND METHODS

The nature and aim of the investigation was explained to the parents and all gave their consent.

Clinical data on the 25 mothers and their infants are given in Table 1. The mean age of the mothers was [illegible]7.1 years (range 19–34 years). All mothers were healthy, their pregnancies were uncomplicated and all were delivered at term. Two mothers (cases 3 and 11) were delivered by elective caesarean section with lumbar anaesthesia because of contracted pelves. In case 11 the anaesthesia was complicated by a transcient drop in maternal systolic blood pressure to 60 mm Hg immediately before delivery. The remaining mothers had vaginal deliveries. Nitrous oxide was not administered to any mother during delivery because the postnatal pulmonary elimination of nitrous oxide by the infants would interfere with the determination of pulmonary gas exchange (53).

Four mothers were given pudendal nerve block (0.5% Citanest®, AB Astra, Sweden, total 10–20 ml). Six mothers received pethidine during labor (Table 1). Twelve mothers were given intravenous oxytocin infusion (20 IE oxytocin/1 000 ml of a solution containing 5% fructose and 5% glucose at a rate of 30 ml/hour). The indications were slight secondary hypotonic uterine inertia (cases 13, 22 and 24, oxytocin was given only during the last hour of labor), Rh sensitization (cases 15 and 28, both infants were Rh negative) and in order to avoid postmaturity (case 27).

All labors and deliveries were carefully observed and fetal heart rate was monitored intermittently using an ultrasonic technique (53). Fetal bradycardia (below 100 beats/min between contractions) was registered in 7 cases during the second stage of labor and in 5 of these infants a nuchal cord was found, in one of them (case 14) the amniotic fluid was meconium stained. Nuchal cords were also found in cases 25 and 26 (Table 1). Despite the existence of these prenatal complications 24 infants were clinically normal at birth and had Apgar scores of 7 or more at 1 min and 10 at 5 min after birth. One infant

Table 1 *Clinical data on mothers and infants*

Group	Case no	Sex	Parity	Duration of: Labor (hours[a])	Duration of: Sec stage (min)	Oxytocin admin	Analgesia: Pud nerv block	Analgesia: Pethidine[b] 1-3 5 hours	Analgesia: Pethidine[b] >3 5 hours	Fetal brady cardia	Mode of delivery presentation	Cord around the neck	Gest age[c] (weeks)	Birth weight (g)	Length (cm)	T_E[d] (C)
	3	♀	II								Caes sect		39	3 050	50	34 6
	7	♀	I	12	40		+				Vertex		40	4 255	55	34 8
	12	♂	III	2 5	5			+			Vertex		40	3 580	52	34 7
	17	♂	III	6	20			+			Occiput post		38	2 770	49	34 8
I	19	♂	III	3	5						Vertex		40	3 580	52	34 7
	23	♀	I	7	50			+			Vertex		40	3 200	50	33 9
	25	♂	II	6*	5						Vertex	+	40	3 820	50	33 4
	26	♂	II	3*	10						Vertex	+	39	2 960	50	33 2
	28	♂	IV	5	5	+					Vertex		40	3 120	48	33 5
	29	♀	III	3*	5						Vertex		40	4 040	52	33 3
II	11	♂	II								Caes sect		39	2 980	49	34 7
	14	♂	I	9	10				+	+	Vertex	+	41	3 580	50	34 5
	15	♂	II	4	20	+		+		+	Occiput post	+	40	2 980	49	31 8
	22	♀	I	12	40	+		+	+	+	Vertex	+	40	2 900	49	33 6
	24	♂	II	14*	20	+			+	+	Vertex	+	41	3 890	54	33 5
	27	♂	IV	7	10	+				+	Vertex	+	42	3 680	50	33 3
III	9	♀	V	3*	5						Vertex		39	3 370	51	32 0
	13	♀	II	5	10	+					Vertex		39	3 320	52	31 7
	16	♂	IV	4	10				+		Vertex		41	4 080	53	32 0
	18	♀	I	7*	20		+				Vertex		39	2 930	48	28 8
	20	♀	III	4 5*	10						Occiput post		41	2 660	48	28 7
	21	♀	I	17*	60		+		+		Vertex		40	3 030	50	29 8
See text	1	♀	I	8	20		+	+			Vertex		40	3 790	53	33 8
	2	♀	I	8	60			+		+	Vertex		40	3 570	52	34 1
	5	♂	I	15	—			+	+	+	Vertex		40	3 620	51	34 0

[a] Duration of labor was the time elapsing from cervical dilation of 2 cm to delivery or estimated from parturiogram (*)
[b] Pethidine 50-100 mg was administrated within 1-3 5 hours or more than 3 5 hours before delivery
[c] Gestational age was calculated in completed weeks from the date of the first day of the last menstruation
[d] T_E = environmental temperature

Table 2 *Comparison between umbilical venous (UV) and arterial (A first sample drawn mean sampling time after birth was 1 4 min) values for FFA glucose β hydroxybutyrate and lactate in 16 infants*

		Mean±SD	P_t	r	P^b
FFA (mM)	UV A	0 262±0 072 0 204±0 082	<0 01	0 71	<0 01
Glycerol (mM)	UV A	0 0735±0 0192 0 0691±0 074	n s	0 57	<0 05
β-Hydroxy butyrate (mM)	UV A	0 314±0 191 0 200±0 133	<0 001	0 92	<0 001
Lactate (mM)	UV A	3 85±1 35 4 57±2 01	<0 05	0 84	<0 001

P_t = degree of significance between UV and A values using paired *t* test
[b] *P* degree of significance of the correlation coefficient (*r*) between UV and A values

trisodium citrate) drawn at 120 min of age In an additional 6 cases lipoprotein lipase activity was determined at the end of the study during the third hour after birth before and 10 min following a single intravenous dose of heparin (500 units in 1 0 ml of 0 15 M sodium chloride solution) The samples were also analyzed for glucose FFA glycerol and β hydroxybutyrate Plasma post heparin lipoprotein lipase activity was assayed *in vitro* according to the method described by Boberg & Carlson (8) except that FFA was analyzed by a micromethod (23)

Study protocol

Immediately after birth the upper airways of the infant were carefully cleared of mucus by suction One group of infants, predetermined for observations at a high environmental temperature were dried with warm (39 C) towels and were kept below a radiant heat source Another group of infants predetermined for observations at a lower environmental temperature were dried with towels (22 C) and were kept in room temperature during the first minutes after birth Within the first minutes after birth the following procedures were performed

1 A catheter was inserted through an umbilical artery into the abdominal aorta and the first blood sample was drawn The umbilical vein was punctured and a blood sample was drawn The cord was clamped 1 min after birth

Simultaneously a face mask was applied and gas collection for determination of the pulmonary gas exchange was begun (53)

3 An electrode was attached to the infant s chest and recording of heart rate was started

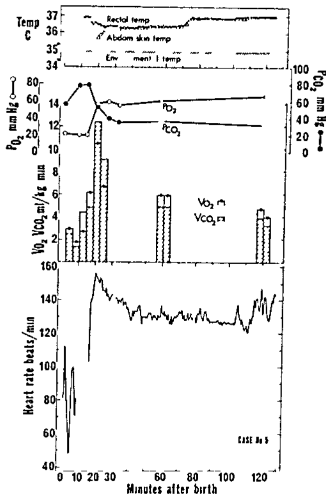
4 A thermocouple was placed 4-5 cm deep into the rectum for rectal temperature monitoring

The above procedures were usually completed within 4 to 5 min of age and the baby was thereafter transferred to a thermocontrolled chamber (53) The baby was kept in the chamber during the first 2 hours after birth The mean environmental temperature of the group of infants who were predetermined for observation at a high environmental temperature was 34 0 C (range 33 2–34 7 C) In the other group of infants which was studied at a lower environmental temperature the mean temperature was 30 5 C (range 28 7–32 1 C) Skin temperatures were recorded at six different sites at 20 min intervals. Rectal and abdominal skin temperatures three different environmental temperatures and heart rate were continuously recorded (53) Changes in motor activity were subjectively evaluated according to a four point scale 0 = infant asleep no movement of limbs 1 = infant awake movement of one limb 2 = infant awake movement of more than one limb 3 = infant crying all limbs moving The mean activity during each 4-minute period of gas collection was calculated

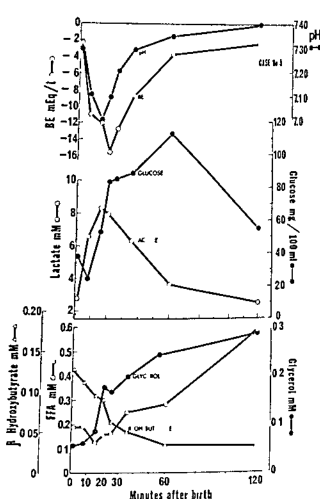
Clinical observations during labor and delivery time of birth condition of the baby motor activity and the times of samplings were tape recorded Blood samples were drawn at predetermined intervals during the first 2 hours after birth for subsequent determination of blood gases acid base balance glucose FFA glycerol β hydroxybutyrate and lactate Determination of the pulmonary gas exchange was performed continuously during the first 20 min after birth in successive 4 min intervals and during two 4 min intervals each at 60 and 120 min of age The different parameters recorded in relation to time are illustrated by a typical case in Fig 1

RESULTS

Simultaneous umbilical venous and arterial blood samples were obtained in 16 infants (Table 2) There were significant correlations between umbilical venous and arterial concentrations for FFA glycerol β hydroxybutyrate and lactate The umbilical venous concentrations for FFA and β hydroxybutyrate were significantly higher than in arterial blood The



a



b

Fig 2 Postnatal asphyxia (case 5 Table 1) Uncomplicated term delivery pethidine was given 15 5 and 2 5 hours before delivery (100 100 75 mg) Apgar core 5 at 1 min No fetal bradycardia was registered and initial values for pH Pco and BE were normal Irregular shallow breathing after birth was associated with very low pulmonary gas exchange values and bradycardia After a period of intensive crying normalization of pulmonary gas exchange and blood gases occurred

(case 5) developed postnatal asphyxia (see legend to Fig 2) All infants had birth weights and lengths within the normal range for their sex and gestational age (49)

Blood collection and analytical procedures

A 90 cm 5 French polyvinylchloride catheter was used and blood samples (2 ml each) were taken in heparinized plastic syringes at predetermined intervals during the first 2 hours of life (Fig 1) The total amount of blood drawn from each infant never exceeded 18 ml A portion of each blood sample was immediately transferred to a gastight glass syringe and stored in ice for later measurement of pH Po and Pco (30) The remaining blood was placed in chilled plastic tubes and stored on ice Within 60 sec after sampling a 200 μl aliquot of the blood was transferred to 1 0 ml of 0 6 M ice cold perchloric acid for lactic acid determination (45) After centrifugation at 4 C of the remaining blood plasma was separated and frozen at −20 C until analyzed in duplicate for glucose (50) FFA (23) glycerol (24) and β hydroxybutyrate (39) The analytical errors were for lactate ±2 4 glucose ±4 1 FFA ±5 5 glycerol ±2 2 and β hydroxybutyrate ±1 9

Between sampling the volume of the catheter (0 75 ml) was filled with 0 15 M sodium chloride solution The sampling procedure was as follows the sodium chloride solution was withdrawn the catheter was repeatedly flushed with arterial blood 2 5 ml of blood was withdrawn and saved a 2 0 ml sample was taken the 2 5 ml blood was re injected and the volume of the catheter was refilled with sodium chloride solution

In 2 cases numbers 1 and 2 minute amounts of a dilute heparin 0 15 M sodium chloride solution (Heparin AB Vitrum Stockholm 50 units/ml sodium chloride solution) were used to fill the catheters between samplings In these 2 cases lipoprotein lipase activity was determined on samples (0 9 ml of blood collected in a syringe containing 0 1 ml 0 1 M

0 65 mM to 0 377 and 0 90 mM respectively The individual concentrations for lipoprotein lipase activity were significantly correlated to the individual increases in FFA concentrations ($r = 0\,81$ $n = 6$ $p < 0\,001$) No significant change occurred in glucose concentrations

Results obtained in case 5 who developed postnatal asphyxia are given in Fig 2

The complete results of changes in pulmonary gas exchange blood gases and acid base balance skin and rectal temperatures heart rate and motor activity in these infants will b published separately (53)

Influence of prenatal history

The possible influence of fetal distress duration of labor and analgesia on the pattern of postnatal changes of FFA and glycerol were analyzed

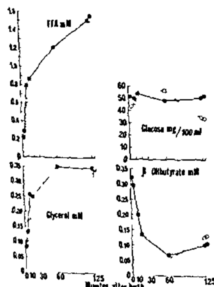


Fig 3 Plasma concentr tions for FFA, glycerol, glucose and β hydroxybutyrate in two infants (cases 1 and 2, T ble 1) The catheters used for blood sampling had been flushed with a dilute heparin saline olution. *Case 1* (●) De eloped fetal bradycardia late in second stage of labor At 1 min after birth th re was se ere a idosis with pH 7 0 P_{CO_2} 95 mm Hg BE 17 mE l and blood lactate 8 mM Subsequent clinical course uneventful. *Case 2* (○) Term delivery (Table 1) Apgar cores 8 and 10 at 1 and 5 min Blood la tate 3 97 mM at 4 min clinical course uneventful.

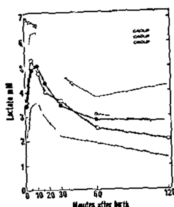


Fig 4 Mean lactate concentrations for the different groups The shaded area represents ±1 SD for group I

Fetal distress Ten infants without signs of prenatal complications were grouped together (group I Table 1) All five infants with a history of fetal bradycardia *during the second stage* of labor and one infant delivered by caesarean section which was complicated by a drop in maternal blood pressure were grouped *together* (group II Table 1) Groups I and II were studied in similar environmental temperatures (*Table 1*)

Group II had a significantly higher degree of acidosis during the first minutes after birth *as compared to group I* The mean levels for pH were 7 15 and 7 30 for base excess −9 6 and −2 6 mE/l and for P_{CO} 69 and 54 mm Hg *in groups II and I respectively* The higher degree of acidosis in group II as compared to group I corresponded to a statistically higher

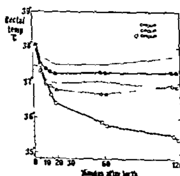


Fig 5 Mean rectal temperatures for the different groups Shaded area represents ±1 SD for group I

Table 3 *Mean values ±S D for sampling times and plasma levels of glucose, FFA glycerol, β hydroxybutyrate and lactate in 22 infants (groups I+II+III)*

Parameter	Sampling times minutes after birth						
	1±0.6	5±0.8	10±1.0	17±2.4	30±0.9	60±1.0	120±1.5
FFA (mM)	0.184 ±0.074 (19)	0.204 ±0.083 (20)	0.202 ±0.090 (20)	0.289 ±0.186 (22)**	0.294 ±0.199 (22)*	0.547 ±0.509 (22)**	0.757 ±0.383 (22)***
Glycerol (mM)	0.0742 ±0.0336 (20)	0.0898 ±0.0390 (19)**	0.1135 ±0.0553 (20)***	0.1488 ±0.0678 (22)***	0.1773 ±0.0755 (22)***	0.2421 ±0.1172 (22)***	0.2782 ±0.0979 (22)***
Glucose (mg/100 ml)	59.4 ±26.1 (19)	63.1 ±28.1 (22)	64.2 ±22.3 (22)	69.3 ±30.8 (22)*	58.0 ±23.3 (21)	56.5 ±22.9 (20)**	59.0 ±14.5 (21)
β Hydroxybutyrate (mM)	0.1758 ±0.1333 (19)	0.1445 ±0.1085 (19)***	0.1098 ±0.0754 (20)***	0.0879 ±0.0624 (22)***	0.0642 ±0.0393 (22)***	0.0643 ±0.0659 (22)***	0.0742 ±0.0673 (21)***
Lactate (mM)	4.55 ±2.06 (20)	5.74 ±1.94 (21)***	5.52 ±1.73 (22)***	4.79 ±1.55 (22)	3.88 ±1.24 (22)	2.83 ±0.93 (22)***	2.52 ±1.29 (22)***

Figures within parenthesis denote number of observations
Significant difference between values at 1 min and values at subsequent sampling times using the paired t test are given as * = $p<0.05$ ** = $p<0.01$ *** = $p<0.001$

arterial concentrations for lactate were higher than those in venous blood

Mean values ± standard deviations for sampling times and for arterial concentrations of FFA glycerol β hydroxybutyrate and lactate obtained in 22 infants during the first 2 hours after birth are given in Table 3 The degree of statistical differences (using the paired t test) between concentrations at 1 min and all subsequent concentrations are given in Table 3 A significant rise in plasma FFA-concentrations was found at 17 min and there after there was a progressive increase A significant rise in plasma glycerol concentration was seen already at 5 min and was followed by a progressive increase The glucose concentrations remained essentially unchanged during the observation period The mean concentra tions of β-hydroxybutyrate showed an initial rapid decrease with time After a significant increase in the mean lactate concentration be tween 1 and 5 *min*, *there was a progressive decrease with time*

The logarithms of *the individual concentra*tions for lactate and β hydroxybutyrate were plotted against time and from the extrapolated straight line formed the half times ($t_{1/2}$) were graphically determined The mean $t_{1/2}$ values ± standard deviations for lactate and β hy droxybutyrate were 36.1±19.6 ($n=15$) and 19.1±11.2 ($n=20$) minutes respectively No relation was found between the highest level of β hydroxybutyrate or lactate and the cor responding rise in FFA or glycerol between 1 and 120 min

Individual concentrations for FFA glycerol, β hydroxybutyrate and glucose in the 2 cases (1 and 2) who received heparin saline solution are given in Fig 3 A distinct increase in con centrations of glycerol and FFA was already seen at 5 min Lipoprotein lipase activities at 120 min were 0.045 and 0.036 μmoles FFA per minute and ml plasma in case 1 and 2 re spectively

In six infants the mean lipoprotein lipase activity increased from 0 at *120 min to* 0.084 μmoles FFA per minute and ml plasma 10 min following heparin administration The mean concentrations for glycerol and FFA increased significantly (paired t test) from 0.343 and

0 65 mM to 0 377 and 0 90 mM respectively The individual concentrations for lipoprotein lipase activity were significantly correlated to the individual increases in FFA concentrations ($r=0\ 81$ $n=6$ $p<0\ 001$) No significant change occurred in glucose concentrations

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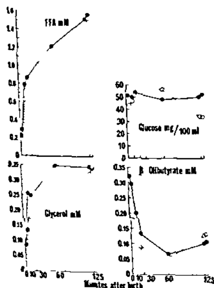


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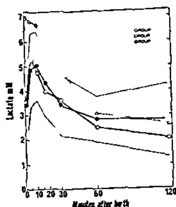


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Fetal distress Ten infants without signs of prenatal complications were grouped together (group I Table 1) All five infants with a history of fetal bradycardia during the second stage of labor and one infant delivered by caesarean section which was complicated by a drop in maternal blood pressure were grouped together (group II Table 1) Groups I and II were studied in similar environmental temperatures (Table 1)

Group II had a significantly higher degree of acidosis during the first minutes after birth as compared to group I The mean levels for pH were 7 15 and 7 30 for base excess −9 6 and −2 6 mE/l and for P_{CO} 69 and 54 mm Hg in groups II and I respectively The higher degree of acidosis in group II as compared to group I corresponded to a statistically higher

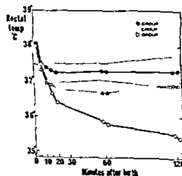


Fig 5 Mean rectal temperatures for the different groups Shaded area represents ±1 SD for group I

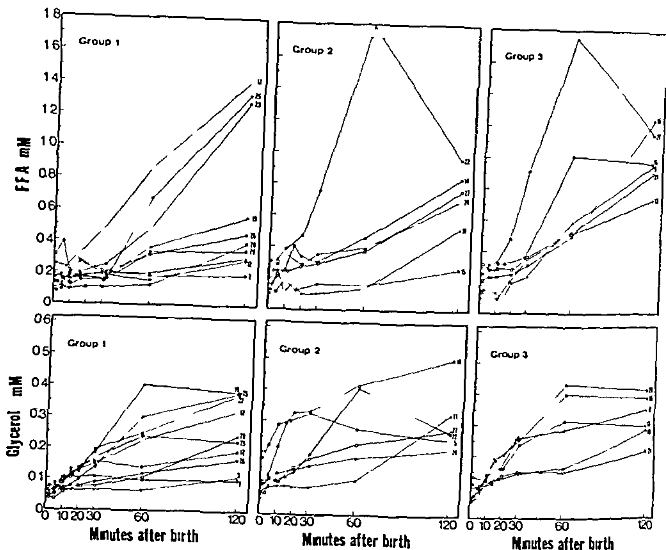


Fig 6 Individual plasma concentrations of FFA and glycerol in the groups I II and III (from left to right)

mean lactate concentration during the first 30 min after birth (Fig 4) Group II had significantly lower mean rectal temperatures during the first 60 min after birth with the lowest mean level at 60 min (Fig 5) During the initial minutes after birth group II also had a somewhat lower mean heart rate as compared to group I (53) The mean levels and the pattern of changes in postnatal pulmonary gas exchange were similar in both groups (53)

No definite difference in the initial levels and the patterns of changes of FFA were detectable when comparing the two groups (Fig 6) The initial glycerol concentrations were slightly higher in group II at 1 and 5 min ($p<0.05$) and the slope of the rises were steeper during the first 30 min (Fig 6) A greater variation in the increase in glycerol concentration was seen in group I The mean concentrations for glucose and β hydroxybutyrate were not significantly different in the two groups (Figs 7 and 8)

The influence of postnatal asphyxia on concentrations of FFA and glycerol are illustrated in Fig 2 The pattern of changes in FFA and glycerol concentrations did not differ from those seen in groups I and II

Duration of labor The highest initial concentrations for β hydroxybutyrate were found in infants of primigravidas There was a positive correlation between the duration of labor in hours and the arterial concentrations (at 1 min) of β-hydroxybutyrate ($r=0.80$, $n=19$ $p<0.001$) and FFA ($r=0.70$, $n=23$, $p<$

0 001) In contrast no such relationship existed between duration of the 2nd stage of labor in minutes and the concentrations of FFA and β hydroxybutyrate No relation was found between the rise in concentration of glycerol or FFA (between 1–120 min) and the duration of labor or second stage

Analgesia Pethidine administration to the mothers during labor was not accompanied by demonstrable differences in initial concentrations or patterns of changes for FFA or glycerol

Influence of environmental temperature

Six infants were studied at a low environmental temperature (group III Table 1) and their results have been compared with those in 10 infants which were studied at a high environmental temperature (group I Table 1) The mean values for rectal temperatures in group III were significantly lower already at 10 min after birth and the lowest recorded mean rectal temperature was 35 4 C at 120 min (Fig 5) During the observation period group III tended to have higher values for motor activity and lower values for P_{CO} (53) The two groups were similar in the following respects prenatal history duration of labor oxygen uptake and carbon dioxide elimination, and heart rate (53)

The mean concentrations for β hydroxybutyrate lactate and glucose were not significantly different in the two groups (Figs 4 8 and 7)

In group III there was an earlier and more

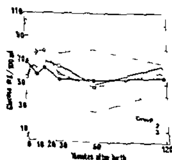


Fig 7 Mean plasma concentrations of glucose in the different groups. Shaded area represents ±1 SD for group I.

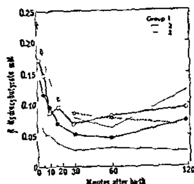


Fig 8 Mean plasma concentrations of β hydroxybutyrate in the different groups Shaded area represents ±1 SD for group I

consistent rise in plasma glycerol and FFA concentrations than in group I

However when the two groups were combined no relationship was found between the individual values for environmental temperatures and the rises in FFA or glycerol between 1 and 120 min or between 1 and 30 min

Relation between plasma FFA or glycerol and other parameters

Glucose No correlation was found between paired concentrations for glucose and FFA or glycerol concentrations in the total material When the lowest glucose concentration in each infant found at 30 or 60 or 120 min was related to the FFA-concentrations at 120 min, no correlation was found Five infants had one or more glucose concentrations equal to or below 30 mg/100 ml When comparing the increment rises in glycerol or FFA between 1 and 120 min between these 5 infants and the remaining 17 no significant differences were found

Motor activity A significant relationship was found between the degree of motor activity and oxygen uptake (53) The rises in FFA or glycerol concentrations (1–120 min) however were not related to the individual means of motor activity for 1–20 or 60 and 120 min.

Respiratory exchanges ratio ($R = V_{CO_2}/V_{O_2}$) The R values were significantly related to the

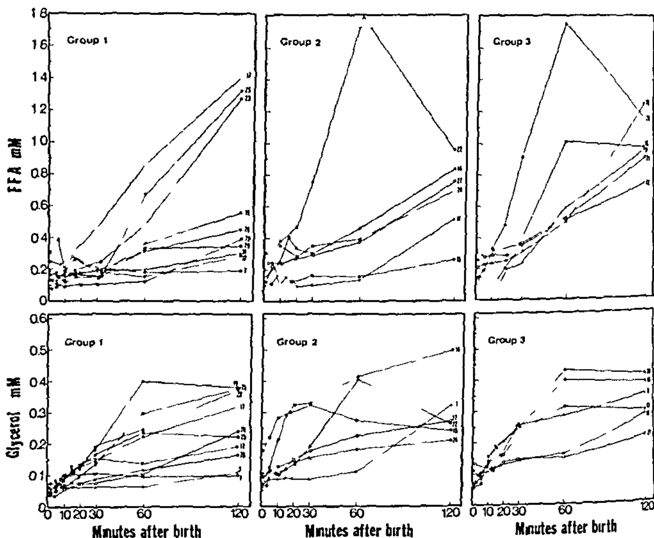


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half hour after birth FFA is either oxidized or re esterified within adipose tissue

In 21–35 day-old anaesthetized puppies experimentally induced hypoxemia resulted in acidosis and decreased plasma concentrations of FFA and glycerol (3) However the P_{CO} values of the animals were low and thus the experimental situation differed from neonatal asphyxia which is characterized by stagnant hypoxia and hypercapnic acidosis Unchanged as well as rising concentrations of plasma FFA have been reported in anaesthetized dogs following hypoxia and hypercapnia (15 16 36 46) On the other hand isolated hypercapnic acidosis in anaesthetized dogs inhibited noradrenaline induced lipolysis (40) The postnatal rise in plasma FFA was inhibited in newborn lambs by severe fetal distress and in newborn rabbits by breathing 100 % nitrogen (13 47)

In the present study signs of fetal distress accompanied by acidosis at birth did not significantly influence the postnatal patterns of changes of FFA and glycerol concentrations In the distressed group (group II) the rise in glycerol concentrations tended to be more pronounced during the first minutes as compared to those in the control group (group I) A distinct rise in plasma glycerol concentration was also seen in an infant during a period of postnatal asphyxia (see case 5 Fig. 2) The finding of a lack of inhibition of lipolysis during asphyxia in the newborn human is in contrast to most of the above mentioned experimental results These discrepancies could be due to differences in the severity of distress age or species variations Interpretation of the results obtained in the dog experiments as compared to the present findings is further complicated by the use of anaesthesia in those studies since pentobarbital has been shown to influence the arterial concentrations of FFA (19)

In a pilot study where the infants were handled in the same way and with the same equipment for thermocontrol it was shown that if the environmental temperature was increased above 34 5 C a rise in rectal temperature towards overheating occurred within the first 30 min after birth Since the route for heat elimination of the *fetus* is via the placenta, the rectal and skin temperatures at the moment of birth are equal and approximately 38 C (54) Following birth a temperature gradient of approximately 2 C has to be established between the environment and skin to allow for the necessary heat elimination (1) The mean rectal temperature in group III (mean T_E 30 C) decreased to 35 4 C at 120 min which is comparable to the rectal temperatures reported in infants who have received routine nursery care (35) It has been reported that infants exposed to a low environmental temperature (room temperature) have higher blood lactate levels and a more pronounced acidosis during the first hours after the birth as compared to infants kept in incubators at a temperature between 32–33 C (17) The moderate difference in thermal stimulation between group I (mean T_E 34 2 C) and III in the present study did not result in any differences in blood glucose levels or rate of elimination of postnatal acidosis and lactate This was in good agreement with a recent study using a similar sampling technique and environmental temperature conditions (48)

Lipolysis and lipid mobilization as indirectly reflected by the rises in glycerol and FFA concentrations occurred irrespective of the ambient temperature chosen but these processes seemed to be more pronounced in the thermal stimulated group (group III)

It has been suggested that the decrease in blood glucose concentration after birth could be one explanation for the postnatal increase in lipid mobilization Reported findings of an inverse correlation between blood glucose and plasma FFA concentrations could be taken as evidence for this hypothesis (9 12) No such relationship however has been found in other studies (38) or in the present investigation In addition the rising glycerol concentrations starting already within the first minutes after birth suggests that lack of available glucose is

Table 4 *Correlation between FFA and glycerol values in the different groups (see text) at different times after birth*

	Age in minutes					
	1–17			30–120		
	r	*n*	*p*	*r*	*n*	*p*
Group I	0 38	34	<0 05	0 55	30	<0 001
Group II	0 32	39	n s	0 72	37	<0 001
Group III	0 20	24	n s	0 70	20	<0 001
Group I+II+III	0 44	97	<0 001	0 67	87	<0 001

r = correlation coefficient
n = number of observations
p = degree of statistical significance

degree of acidosis (53) At 120 min after birth when the acidosis had been eliminated in all infants, the mean R-values ± S D for group I, II and III were 0 88 ± 0 065, 0 85 ± 0 054 and 0 77 ± 0 076 respectively An inverse correlation was found between the rise in FFA (between 1–120 min) and the R-value measured at 120 min ($r = 0\ 47$ $n = 24$, $p < 0\ 05$)

Relation between FFA and glycerol The relationship between FFA and glycerol concentrations was calculated during two time intervals between 1 and 17 min and between 30 and 120 min (Table 4) A relationship between FFA and glycerol concentrations in all groups was found only during the latter time interval

DISCUSSION

The importance of not using heparin solutions for catheter patency is illustrated by the present results, confirming previous reports that heparin activated lipoprotein lipase in the newborn human (20) Furthermore minute amounts of heparin induce significant changes in plasma concentrations of FFA and glycerol

The differences in reported mean cord blood FFA concentrations, ranging between 0 1 to 0 6 mM in different studies (9 12 21 22 29, 38, 42 51), probably reflect systematic differences in analytical techniques

The results of previous studies on the influence of fetal distress during labor on FFA concentrations in umbilical cord blood are conflicting and both elevated and low levels have been reported (21, 41, 42, 51) The arterial FFA concentrations at 1 min in the present study were not significantly different between infants with or without signs of fetal distress during labor In accordance with recent reports (44) we found slightly higher initial glycerol concentrations in the group with signs of fetal distress (group II) In addition, both initial FFA and β hydroxybutyrate concentrations were significantly related to the duration of labor

Our finding of no significant arterio-venous difference for glycerol concentrations in cord blood was in agreement with previously reported results (44) In contrast to one (43) but in agreement with other reports (9, 1?), were the higher concentrations of FFA in umbilical venous rather than arterial blood Unlike these studies however, this arterio venous difference was significant, but only when the paired *t* test was used Since concentrations of some parameters measured in cord blood samples seem to be only representative for the situation at birth being influenced by the process of labor and delivery, reported estimations of fetal energy metabolism based on cord arterio venous differences (43) must be interpreted with extreme caution

The results of the present study clearly indicate that almost immediately after birth an increase in lipolysis occurs as evidenced by the rapid rise in glycerol concentrations In contrast the rise in FFA concentrations was generally delayed until between 30 and 120 min after birth The initial low concentrations of FFA indicate a suppressed lipid mobilization In agreement with this finding are recent *in vitro* studies on isolated fat cells from newborn human infants which have shown a high release of glycerol and a low outflow of FFA to the medium as compared to adipose tissue from older infants and adults (34) Our results would therefore indicate that within the first

been eliminated an inverse correlation was found between the rise in FFA from birth to 120 min and the respiratory exchange ratio (V_{CO_2}/V_{O_2})

The glucose concentrations were related neither to the FFA nor to the glycerol concentrations The rate of elimination of lactate and β hydroxybutyrate was not influenced by environmental temperature or acidosis Minute amounts of administered heparin caused an increased rise in FFA and glycerol concentrations which were associated with the appearance of lipoprotein lipase activity

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a very unlikely explanation for the increase in lipolysis

During labor the maternal concentrations of blood ketones increase and at delivery a significant relationship exists between maternal venous and cord blood concentrations (37, 39, 48) In agreement with previous studies are the present findings of a higher concentration of β hydroxybutyrate in umbilical venous than in arterial blood and the significant correlation between venous and arterial concentrations (37, 43) The highest concentrations of β hydroxybutyrate were found at 1 min after birth but they did not reach the reported concentrations known to inhibit lipolysis (6)

No relationship was found between concentrations of β-hydroxybutyrate and postnatal changes in FFA and glycerol concentrations The hypothesis that the cord concentrations of ketones are mainly determined by the ketone body infusion from the mother to the fetus and not by the ketone body production by the fetus is supported by the present findings of a very rapid postnatal elimination rate of β-hydroxybutyrate This rapid elimination of β-hydroxybutyrate was found in all infants irrespective of their condition at birth and their environmental temperatures

During the first hour after birth, the respiratory exchange ratio primarily reflects the elimination of combined acidosis (53) It is thus impossible to draw any conclusions from the values of respiratory exchange ratio (R) concerning *tissue* metabolic respiratory quotient (RQ) during this time period At 120 min after birth the arterial blood pH and lactate values were normalized and at this time R values could represent RQ The findings of an inverse relation between the rise in FFA during the first 2 hours after birth and the R values at 120 min support this view The significantly lower R-values at 120 min present in group III (T_E 30 5°C) as compared to group I (T_E 34 0°C) might indicate differences in the relative degree of oxidation of fat between these two groups of infants However, the short registration periods (4+4 min) for the determinations of R values do not allow any definite conclusions concerning the possible influence of different ambient temperatures on the degree of oxidation of fat in the newborn infant

Although various factors (27,38) may influence lipolysis and lipid mobilization in the newborn, the strikingly rapid rise in glycerol concentrations during the first minutes after birth suggest that during this period the dominant factor controlling lipolysis is an increase in sympathetic nervous activity In the normal fullterm infants of this study, the results furthermore suggest that the effect of environmental temperature and acidosis on the increase in lipolysis were marginal The lack of an inhibiting influence on lipolysis and lipid mobilization of these factors is probably of great importance for the energy metabolism of the newborn

SUMMARY

Arterial concentrations of glycerol FFA, glucose lactate and β hydroxybutyrate were serially measured during the first two hours after birth in normal fullterm infants in a thermo controlled environment. Blood gas tensions, acid base balance pulmonary gas exchange, motor activity and heart rate were also determined a detailed report of these data will be published separately In 22 infants the glycerol concentrations showed a rapid immediate increase after birth wheras the rises in FFA concentrations were delayed until between 30 and 120 minutes indicating a prompt increase in lipolysis and a suppression of lipid mobilization during the first half hour after birth This suppression might be explained by a high rate of re esterification or oxidation of FFA within adipose tissue The influence of environmental temperature (28 7–34 8 C) and degree of acidosis on the pattern of changes in FFA and glycerol were only marginal No inhibition of lipolysis and lipid mobilization was shown in an infant who developed postnatal asphyxia

At 120 min after birth, when acidosis had

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THE DIAGNOSIS OF TAY SACHS DISEASE[1]

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Tay Sachs disease is an autosomal recessive inherited disorder characterized by widespread deposits of a specific ganglioside G_{M2} in the neurons but also to some extent in the glial cells Earlier work in this laboratory showed that this ganglioside differs from the major brain gangliosides by its carbohydrate moiety in that it consists of only three neutral sugar moieties leaving *N* acetylgalactosamine in end position of the chain (25) A storage of this ganglioside was later demonstrated in some visceral organs (3) On the basis of this finding a generalized defect of a β *N* acetyl galactosaminidase degrading the G_{M2}-ganglioside was predicted as the enzymatic failure in Tay Sachs disease (3)

Recent investigations on hexosaminidases in mammalian tissues (1 18) have produced new information of importance for the further study of the enzymatic lesion in Tay Sachs disease The hexosaminidases of normal mammalian tissues can be subfractionated into two major fractions A and B by electrophoresis ultracentrifugation or column chromatography Okada & O Brien (15) who used starch gel electrophoresis showed that the hexosaminidase fraction A was missing in brain liver kidney skin and white blood cells in patients with classical Tay Sachs disease Recent research has also produced evidence for more than one genetic form of a given gangliosidosis with a different enzymatic lesion (2 21 31)

The diagnosis of a ganglioside storage disease such as Tay-Sachs disease thus requires the identification of the accumulated substance and characterization of the enzymatic failure The aim of the present study was to find a tissue suitable for determination of the ganglioside deposit in biopsy specimens and to develop a simple method for analysis of the hexosaminidase pattern

MATERIALS

Tissues were available from a 3 year-old girl and a 4-year-old boy who had died from Tay Sachs disease Control tissues were obtained from 20 subjects who had died of diseases not involving the central nervous system Tissue was also available from one patient with globoid cell leukodystrophy (GCLD) from one patient with late infantile metachromatic leukodystrophy (MLD) from two patients with juvenile amaurotic idiocy (JAI) and from one patient with multiple sclerosis (MS)

A detailed case report of the 4-year old boy with Tay Sachs disease has been published earlier (3) The other patient who died from Tay Sachs disease at the age of three years also belonged to a non Jewish family with no earlier family history of the disease The first symptoms of weakness and delayed motor development were noticed at the age of 6–7 months The disease ran a characteristic course and from the age of 6 months the child assumed a permanent posture of decerebrate rigidity" She died at 36 months from aspiration pneumonia.

METHODS

Lipid determinations Total lipids were extracted with chloroform methanol (1 1) v/v) and phospholipids

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Table 2 *Fractionation of glycosidase activity by ultracentrifugation with a patient with Tay–Sachs disease and a control*

Tissue samples for the analyses were taken from the cortex of frontal lobe The brains were stored at −20 for 9 months before analysis Ultracentrifugation and assay of glycosidase activity was performed as described in "Methods" The enzyme activities are given under A as nmoles *p*-nitrophenol released per hour per mg enzyme protein and under B as percent of homogenate activity

Brain tissue	β-glucosidase		β-galactosidase lactate pH 3 6		β N a etylgalactos amınidase		β-N acetylglucos-aminidase	
	A	B	A	B	A	B	A	B
Tay Sachs patient (3 0 y)								
Homogenate	168	100	88	100	305	100	1 732	100
100 000 g, sediment	213	92	62	51	387	100	2 373	99
100 000 × g, supernatant	33	2	214	29	28	1	257	2
Control (3 5 y)								
Homogenate	36	100	44	100	47	100	500	100
100 000 g, sediment	29	67	26	49	22	38	234	38
100 000 × g, supernatant	0	0	117	39	214	65	2 248	64

of that in liver The ganglioside pattern was more complex and only about 75% of the total amount of *gangliosides* could be iden tified G_M–NAN constituted 33–50 % of total ganglioside-NAN

A typical nerve ganglioside pattern was found in the lipid extracts of the muscular and mucosal layers of the rectum G_M constituted 80 and G_{M1} and G_{D1} predominated among the other gangliosides The ganglioside con centration was about three times higher in the muscular layer than in the mucosa

In n rvous tissue from the two subjects with Tay Sachs disease one oligohexoside asialo-ganglioside G_{A2} was demonstrated in appreci able amounts Its concentration in the two subjects was 3% and 6% respectively of that of G_{M}

The hexosamine-containing glycolipids were estimated in the spleen liver and kidneys of controls and in the two cases of Tay Sachs disease The asialogangliosides G_{A1} and G_{A2} could not be demonstrated in these organs but the major glycolipid had the same com position and migration rate at chromato graphy as globoside Its concentration was 0 40–0 80 µmoles/g fresh tissue in the two cases of Tay Sachs disease

Glycosidase activities in brain tissue The ac tivities of β glucosidase β-galactosidase β N acetylgalactosaminidase and β N acetylglucos-aminidase were assayed in the brain tissue from the 3-year-old girl with Tay Sachs dis-ease To estimate the effect of long storage of the brain material on the enzyme activities tissue samples were assayed fresh and after storage at −20° Storage caused a decrease in glucosidase activity but an increase in hex osaminidase activity The changes did not however essentially affect the profile of gly cosidase activities of the control and patho-logical material. Similar enzyme activities were obtained with 0 32 M sucrose and dis-tilled water as homogenization medium for the tissue

The *p*-nitrophenyl glycosidase activities in the cerebral cortex of the 3-year-old girl with Tay Sachs disease were 5–10 times those of the control material (14) with the exception of β galactosidase (assayed at pH 3 6) which was only moderately increased (Table 2) The glycosidase activities in white matter were about 10 times higher in the girl with Tay Sachs disease than in the control material except for β galactosidase (assayed at pH 5 0) which was only increased 5 times

The cerebral cortical glycosidase activities were not increased in the patients with GCLD MLD JAI and MS In GCLD and MS the glycosidase profile was fairly similar to that

Table 1 *Concentration of gangliosides and phospholipids in various organs of two patients with Tay-Sachs disease*

The figures are expressed in μmoles/g fresh tissue weight Figures in parentheses give the number of controls analysed

Tissue	Phospholipids			Gangliosides		
	Normals (1-5 y)	K K (3 y)	J A (4 y)	Controls (1-5 y)	K K (3 y)	J A (4 y)
Cerebrum frontal cortex	44 (6)	30	21	1 5 (6)	12 1	5 6
Cerebrum frontal white matter	98 (6)	36	17	0 64 (6)	5 9	4 3
Cerebellum cortex	39 (3)	33	23	1 2 (3)	6 6	6 5
Spinal cord	102 (2)	81	68	0 45 (2)	2 8	3 8
Spinal root	100 (1)	91	59	0 20 (1)	1 2	1 3
Adrenal		40			1 4	
Liver	26 (4)	29	41	0 02 (2)	1 4	0 6
Spleen	16 (4)	22	23	0 12 (2)	1 2	0 7
Lung		15			0 1	
Kidney	19 (4)	19		0 05 (2)	0 2	
Ovarium		15			0 3	
Rectum mucosa		14			0 3	
Rectum muscular layer		11			1 0	

and gangliosides determined as earlier described (26) Individual gangliosides were isolated by chromatography on silica gel G and H (29) and identified by comparison to authentic standards

Enzymatic determinations Ganglioside sialidase and p nitrophenylglycosidases were assayed with the methods described in a previous publication (14)

Electrophoretic separation of brain hexosaminidases in agarose gel Plates for electrophoresis were prepared in the following way 23 ml of a 1% agarose (Behringwerke AG Marburg Lahn Germany) solution in 0 04 M phosphate citrate buffer pH 6 0 heated at 60 for 30 min was cast on a preheated 10 × 20 cm glass plate The plate was aged for 24 hr at 4 in a moist chamber before use The 0 04 M phosphate citrate buffer pH 6 0 was used as electrode buffer Contact between electrode vessels and gel was achieved by cellulose tissues 7 cm apart on the gel Slits for 6 samples were cut in the middle of the plate

Tissue homogenate a 5 (w/v) homogenate of frozen samples of brain tissue was prepared by homogenizing by ten strokes in ice cold distilled water in an all glass homogenizer The homogenate was then frozen and thawed three cycles in solid carbon dioxide ethanol 10-15 μl of each sample was applied to the agarose plate Electrophoresis was performed with a voltage of 30 V/cm for 30 min with cooling Indication of hexosaminidase activity the plate was incubated for 1 hr at 37 in 0 001 M 4 methylumbelliferyl β D 2 acetamido 2 deoxigluco pyranoside (Koch Light Laboratories Ltd Colnbrook England) in 0 1 M citrate buffer pH 4 4 The substrate solution was allowed to drip off and the plate was then gently sprayed with 1 M glycine Na carbonate buffer pH 10 0 The location of fluorescent enzyme active fractions was viewed under a Desaga UV lamp at 366 nm

When normal cerebral cortex was investigated two enzyme fractions were distinguished One moving anodically corresponding to the hexosaminidase fraction A described by Robinson & Stirling (18) and Okada & O Brien (15) The other fraction B migrated slowly towards the cathode

Ultracentrifugation of brain tissue A 5 homogenate was prepared as for the electrophoresis experiments The homogenate was centrifuged at 105 000 g (rotor 40 40 000 r p m) for 1 hr at 0 in a Spinco L ultracentrifuge The supernatant was retained and the sediment reconstituted with distilled water and homogenized

RESULTS

Gangliosides and oligohexosides The concentration of gangliosides was increased 4 to 8 fold in necropsy specimens of the cerebrum and cerebellum in the subjects of Tay Sachs disease (Table 1) The increase of the ganglioside concentration was roughly equal in spinal cord and spinal roots Ganglioside G_{M} constituted about 90% of the total gangliosides in the entire central nervous system

Of the other visceral organs the adrenals the liver and the spleen contained gangliosides in higher concentrations than the other organs examined (Table 1) The ganglioside pattern was not so uniform as in the nerve tissue but two gangliosides G_{M} and G_{M3} predominated In the other visceral organs the ganglioside concentration was only 1/5-1/10

stepwise manner, each step being catalyzed by a presumed specific enzyme (for review see 4 28) with one exception the ganglioside sialidase which does not seem to be specific for any particular ganglioside

In the inherited diseases of lipid metabolism leading to the storage of specific lipids in the tissues *the lipidoses* the known enzymatic defect has always been on the catabolic side Therefore one might postulate that all inherited diseases with a primary storage of a certain ganglioside *the gangliosidoses* are caused by a lesion of a catabolic ganglioside enzyme In normal human brain the first step in the degradation of the major gangliosides G_{D1} and G_{T1} involves the release of a sialic acid (9 13 28) No disease is known in which these gangliosides are accumulated which means that no inherited failure of ganglioside sialidase is known The figure for the ganglioside sialidase activity in the cerebral cortex of one of our cases of Tay Sachs disease was comparatively low but within the normal range for age

The monosialogangliosides G_{M1} G_{M2} and G_{M3} (Fig 1) which are intermediates in the degradation of G_{D1} to lactosylceramide have all been shown to be stored in the respective forms of gangliosidoses These three major forms of gangliosidoses have been named G_{M1} G_{M2} and G_{M3}-gangliosidosis according to the type of ganglioside stored (27)

Theoretically the clinical symptoms and signs and the pathological anatomical picture should be the same irrespective of the type of ganglioside stored Identical or very similar morphological features have been shown in the nervous system (3 7 12 24) The clinical manifestations however differ between the three forms of gangliosidoses but also within a given form

The clinical manifestations of the only patient with G_{M3}-gangliosidosis on record (7) differed from those of classical Tay Sachs disease by massive splenomegaly Histological examination of lymphatic tissue showed a replacement of the normal tissue elements by

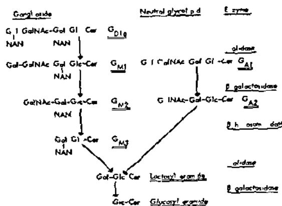


Fig 1 Degradation of gangliosides and corresponding neutral glycolipids of brain Cer = ceramide GalNac = *N* acetylgalactosamine Gal = galactose Glc = glucose NAN = *N* acetylneuraminic acid

foam cells The nervous system contained large deposits of ganglioside G_{M3} and lactosyl ceramide (17)

The onset and clinical course of the most common form of G_{M1} gangliosidosis resemble those of classical Tay Sachs disease (21) but in the former also the viscera and skeleton are involved Because of its generalized nature this form of G_{M1} gangliosidosis has also been called "generalized gangliosidosis (12) It was originally claimed (12) that the storage of G_{M1} ganglioside was responsible for the extraneural manifestations The normal major brain gangliosides (G_{M1} G_{D1} and G_{T1}) and G_M occur only in minute amounts in extraneural organs Yet a 100-fold increase of G_{M1} and G_{M2} may occur (Table 1 24) in the respective gangliosidosis it is not large enough to produce clinical symptoms Suzuki et al (24) later showed extensive storage of two mucopolysaccharides keratan sulfate and a sialomucopolysaccharide An almost complete lack of β-galactosidase activity has been demonstrated both with *p*-nitrophenyl β galactoside and G_{M1} substrates (16) In a more uncommon variant of G_{M1} gangliosidosis (2) the onset is later the course slower and the clinical manifestations dominated by psychomotor retardation and ataxia without any obvious extraneural symptoms In many respects it

Table 3 *Activity of ganglioside sialidase in some disorders affecting brain lipid metabolism*
The assay was performed on frozen specimens of frontal brain cortex

Brain tissue	Age	Sialidase activity nmoles released NAN per mg tissue protein (M±S D)
Controls	0–21 months (n = 8)	2 2±0 7
Controls	3–85 y (n = 21)	6 0±1 1
Tay–Sachs disease	3 y	2 8
Infantile metachromatic leucodystrophy	3 y	3 4
Globoid cell leucodystrophy	1 y	5 1
Juvenile amaurotic idiocy	17 y	5 1
Juvenile amaurotic idiocy	20 y	4 5
Multiple sclerosis	46 y	5 7

in control material In JAI and MLD the hexosaminidase activities were increased 3–5 times

Subfractionation of β glycosidase activities by ultracentrifugation Homogenates of brain cortex from the 3 year old girl with Tay Sachs disease and from a control were subjected to high speed centrifugation The sediment and supernatant fractions obtained were assayed for glycosidase activities (Table 2) In the control material, the β glucosidase activity was exclusively particle bound and was recovered in the sediment About 2/3 of the β N acetylgalactosaminidase and β N acetylglucosaminidase activities were obtained in the supernatant The glycosidases from cerebral tissue from the girl with Tay Sachs disease showed a similar distribution except for the two hexosaminidases both of which were found exclusively in the sediment fraction

Almost all the hexosaminidase activity was found in the supernatant of liver from normals and from the two cases of Tay-Sachs disease The supernatant and the sediment of the muscular layer of the rectum showed only very low hexosaminidase activity

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Brain ganglioside sialidase The activity of ganglioside sialidase was determined in samples of cerebral cortex from the 3 year-old girl with Tay Sachs disease, from GCLD, MLD JAI and MS (Table 3)

The sialidase activity in the TS patient was low and that in the GCLD patient high compared to age matched controls while in the other diseases studied the activities were within the range found for the controls A search was made for the possible presence of inhibitory factors affecting the assay of ganglioside sialidase Mixtures of separately assayed enzymes prepared from normal brain tissue and from pathological material were incubated The following recoveries of enzyme activity was observed Tay Sachs disease 106% MLD 97% JAI 98% and GCLD 95%

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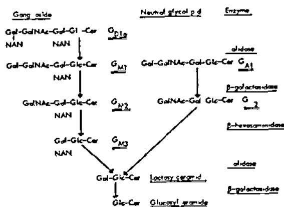


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DISCUSSION

The biosynthesis and biodegradation of brain gangliosides have been shown to occur in a

advantage that the material can be used for microscopical examination

Previous methods for subfractionation of the hexosaminidases (6 15 19) were too complicated and time-consuming for routine determinations In the agarose gel electrophoresis method elaborated in the present study the same equipment and technique were used as for routine serum protein electrophoresis The enzymatic determination can be performed on material from many sources but liver or white blood cells seem to be the most convenient After this study had been concluded an acrylamide gel electrophoretic method was described which has been adopted for the identification of Tay Sachs disease carriers (5) A fluorimetric method has also been elaborated for the assay of hexosaminidase A in serum which may be useful for the detection of homozygotes and heterzygotes for Tay Sachs disease (11)

Tay Sachs disease was originally classified among the amaurotic idiocies but it is now generally accepted to designate a gangliosidosis after the chemical structure of the stored ganglioside as first proposed by Suzuki & Chen (23) A subdivision of the gangliosidoses according to the enzymatic abnormality was recently suggested by one of us (27) and by O Brien (10) variant forms being described as types I II etc A more sophisticated nomenclature for the enzymatic subdivision of the G_{M2}-gangliosidosis has also appeared (31) We consider that a more comprehensive system of enzyme nomenclature should not be used until specific ganglioside substrates have been applied and the relation between the two hexosaminidases and G_{M2} and neutral aminoglycolipids has been established

Young et al. (31) have also discussed the use of the clinical designation Tay Sachs disease Volk (30) has used the term synonymous with amaurotic idiocy and thus included diseases with no disturbances of ganglioside metabolism (27) The other extreme would be to use the name only for the infantile form of G_{M2}-gangliosidosis variant II in which hexosaminidase A is missing and hexosaminidase B is increased (Table 4) Since the three forms of infantile G_{M2}-gangliosidosis apparently produce the same clinical manifestations we consider it justified to use the name "Tay Sachs disease synonymously with infantile G_{M2}-gangliosidosis

SUMMARY

Gangliosides and neutral aminoglycolipids were determined in several organs from two subjects with classical Tay Sachs disease Ganglioside G_{M2} was stored in all organs investigated Of the extraneural organs the highest concentration was found in the adrenals spleen and liver The rectal muscular layer had a high concentration of gangliosides Rectal biopsy might be used for studies of the nervous ganglioside pattern

The brain tissue showed a many fold increase of the activities of *p*-nitrophenylglycosidases The activity of ganglioside sialidase in the brain was not increased in Tay Sachs and some other neurometabolic diseases Subfractionation of the glycohydrolases by ultracentrifugation showed no hexosaminidase activity in the supernatant fraction of the brain in Tay Sachs disease compared with about 2/3 of the total activity in normal human brain

A simple agarose electrophoretic method was developed for the separation of the hexosaminidases into two fractions A and B Hexosaminidase A was virtually absent in Tay Sachs disease

The known forms of gangliosidoses and their biochemical characteristics are reviewed and a scheme is given for their classification and diagnosis

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Table 4 *The classification of the gangliosidoses*

Suggested name	Trivial name	Enzyme abnormality
G_{M1} Gangliosidosis		
Infantile form	Norman-Landing disease Generalized gangliosidosis	G_{M1} and *p*-nitrophenyl *p* galactosidase <10 of normal activity
Late infantile form		*p*-nitrophenyl β galactosidase low
G_{M2}-gangliosidosis		
Infantile form		
Variant I	Tay-Sachs disease	Hexosaminidase A absent Hexosaminidase B absent
Variant II	Tay-Sachs disease (classical form)	Hexosaminidase A absent Hexosaminidase B high
Variant III	Tay-Sachs disease	Hexosaminidase A high Hexosaminidase B high
Late infantile form		Hexosaminidase A low Hexosaminidase B normal
Juvenile form		
G_{M3} gangliosidosis		
Infantile form	Neurovisceral gangliosidosis	

resembles the late infantile form of amaurotic idiocy described by Jansky & Bielchovsky (22)

The biochemical characteristics of patients with classical Tay Sachs disease are the large accumulation of G_{M2} ganglioside in nervous tissue, a many fold increase of G_{M2} in extra neural organs a moderate increase of neutral aminoglycolipid G_A in the brain and little or no increase of globoside in visceral organs The activity of β hexosaminidase in tissues and serum is increased (11 15) but fraction A is missing

Variant forms have recently been described (Table 4) Jatzkewitz and collaborators (20) have described one patient with a clinical record of classical Tay Sachs disease but with transient enlargement of the spleen and liver In that patient the brain content of G_{M2} was the same but G_A was much larger than in the present two cases of Tay Sachs disease An extensive storage of globoside was found in the kidney and histochemical studies revealed signs of glycolipid storage in the spleen and liver The hexosaminidase activity assayed both with the *p* nitrophenyl *N* acetyl β glucosaminide and -galactosaminide and labeled globoside and G_{A2} was only a few percent of normal In a systematic study of hexosaminidases from brains of subjects with a history of all the typical symptoms and signs of Tay-Sachs disease Sandhoff (19) found increased activities of both hexosaminidases A and B in one case Also, a late infantile and a juvenile form of G_{M2} gangliosidosis have been reported In the late infantile form the clinical manifestations were the same as those described by Jansky and Bielchovsky for late infantile amaurotic idiocy (8) The content of G_{M2} ganglioside was almost as high in the brain as in Tay-Sachs disease, and G_{M} was also shown in the liver Hexosaminidase A activity was diminished (31)

The biochemical diagnosis of a gangliosidosis should consequently include the identification of the stored ganglioside and the determination of the enzymatic abnormality The investigation can be performed early in the course of the disease by examination of a biopsy specimen of the brain The present study has shown that also some other organs can be used for identification of the stored ganglioside The second largest relative accumulation of G_{M2} occurred in the liver but the separation of G_{M2} from the normal G_{M3} may be difficult The muscular layer of the rectum has a nerve ganglioside pattern and the chromatographic characterization of the gangliosides is simpler Rectal biopsy has also the

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A CONTROLLED TRIAL ON THERAPY FOR NEWBORNS WEIGHING 750–1 250 g

I Clinical Findings and Mortality in the Newborn Period

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Hitherto few investigations have been devoted solely or extensively to therapeutic procedures capable of decreasing the high mortality rate in newborns with very low weight (14 43 44) The intravenous infusion with glucose and sodium bicarbonate has been shown to improve survival in controlled trials on newborns with respiratory distress syndrome (RDS) weighing more than 1 25–1 50 kg (37 43) This procedure has been advocated routinely also for smaller newborns with or without RDS (3) However the experimental evidence on the benefit of intravenous infusion as compared with oral feeding in very small newborns is still limited and inconclusive In fact in one trial (14) there was some suggestion but no conclusive proof of a decrease in mortality after intravenous infusion whereas no change in neonatal survival was found in another study (43)

Although the reported incidence may vary from one institution to the other infection and hemorrhage are common causes of death in newborns with very low weight (12) This was confirmed in a preliminary investigation in this Centre (31) on a consecutive series of 27 autopsies in newborns weighing 750–1 250 g in this series, the incidence of pneumonia was 26% and the incidence of intracranial hemorrhage 52% Therefore the problem of an effective antimicrobial therapy (18) and of the correction of possible coagulation defects (17) needs to be considered

We were thus led to perform a controlled therapeutic trial in newborns with birthweight of 750–1 250 g Two therapeutic regimens were contrasted one of very active therapy with intravenous infusion with glucose and sodium bicarbonate 3 antibiotics gamma globulins and fresh human plasma (the "treatment series), the other with standard oral feeding and a single broad spectrum antibiotic (the control series) In the present paper, clinical findings mortality data and post mortem observations in the neonatal period will be presented. Laboratory and cardiac observations have been given in another report (11) Follow-up studies are also in progress

MATERIAL AND METHODS

All subjects who met the following requirements entered the trial 1) weight on admission of 750–1 250 g 2) admission, as well as acid base and chest X ray studies, within 24 hours of birth 3) no major congenital malformation or hemolytic disease on first clinical and laboratory examination. Assignment to the treatment or to the control group was performed as follows. Infants were stratified in four subgroups according to their weight and blood pH on admission (weight above/equal or below 1 090 g, and blood pH above/equal or below 7 27—these were the median weight and pH values in a consecutive series of

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Table 1 *Plan of therapy in the treatment and control groups*

	Treatment group	Control group
Fluid and calories	I v infusion 70 ml/kg/day (10 glucose + $NaHCO_3$) for at least 4 days Oral feeding (only pooled human milk) starting in 2nd day of life stepwise increase up to 150 ml/kg/day by 7th–8th day	Oral feeding none on 1st day 10 glucose (25 ml kg/day) from 2nd to 5th day pooled human milk 25 50 75 125 150 175 ml/kg/day from 3rd to 8th day
Antibiotics	Sodium penicillin G 500 000 U + methicillin 100 mg + colistin 8 mg per kg/day for 5–7 days (i v i m after i v stopped)	Kanamycin sulf (20 mg/kg/day i m) in first 5 days Sodium penicillin G (100 000 U i m) after 5th day when required
Other therapies	Gammaglobulin preparation 0 5 ml/day in first 3 days fresh human plasma 3 ml every 2nd day in first 5 days Vitamin K1 (3 mg i m) on admission oxygen when needed (up to 100 conc) ambient temperature 32 –34 C or servocontrol of body temperature	

fants at 2–6 hours in 13 at 7–12 hours in 9 at 13–18 hours and in 3 at 19–24 hours) and it was discontinued between the 4th and the 7th day Oral feeding was started between 22 and 50 hours of birth (with the only exception of 5 infants who were fed at 51–60 hours) In the controls the first feeding was given on the average at 24 5 hours of age (in 2 subjects between 29 and 39 hours and in the remaining subjects between 20 and 28 hours)

The mean amount of fluid and calories given per day to treated and control babies in the neonatal period has been reported on Table 4 The most marked contrast was present in the first 3 days of life when treated infants received almost three times as much fluid and calories than the controls The difference was small in the 4th–5th day and disappeared by the 6th–8th day of birth

Outcome and post mortem findings

The mortality rate in the first 10 days of life in the 80 infants studied was 61 2 % Data on mortality and the most relevant post mortem findings in the experimental groups have been summarized in Table 5 The neonatal survival curve has been illustrated in Fig 1 None of

Table 2 *Some characteristics of infants in the trial*

	Treated	Controls
Number of cases	40	40
No (%) c birthweight < 1 kg	18 (45 %)	17 (42)
No. (%) c gest age < 28 weeks	22 (66 %)[b]	19 (61 %)
No ( ) c weight < 10th perc for gest age[a]	5 (16 %)[b]	3 (10 %)
No ( ) males	17 (42 %)	21 (52 %)

According to the single birth intrauterine growth chart of Lubchenko et al (27)
[a] Known in 32 infants.
Known in 31 infants

Table 3 *Clinical and laboratory findings (mean ± S D or) on admission in infants in the trial*

	Treated	Controls
Number of cases	40	40
Age hours	6 8 ± 5 5	6 5 ± 4 9
Silverman s score	3 8 ± 2.4	3 6 ± 1 8
Respirat rate min	51 1 ± 10 3	55 0 ± 11 0
No (%) c marked X ray abnormality	13 (32 %)	10 (25 %)
SBP mmHg	39 5 ± 5 9	40 9 ± 8 5
Arterial acid–base status		
age of study hours	8 5 ± 5 6	8 8 ± 5.2
pH	7 311 ± 0 140	7 329 ± 0 090
Base Excess mEq l	−7 2 ± 5 5	−7 2 ± 4 0
Pco_2, mmHg	44 2 ± 17 9	37 7 ± 14 2

40 babies with the same weight range, previously admitted to the unit) Within each subgroup subjects were included in the treatment or in the control series by means of sealed envelopes It was established in advance that the trial was to be stopped after studying 40 treated and 40 control infants

The plan of treatment has been summarized on Table 1 Additional explanations are as follows The aim of $NaHCO_3$ administration was to keep the blood Base Excess as near as possible to 0 mEq/l or at most slightly above such level in order to compensate for hypercarbia (but no attempt was made to completely compensate for severe hypercarbia) For this purpose an initial dose of $NaHCO_3$ (mEq given = blood Base Deficit mEq/l × body weight, kg × 0 5) was given in 2–6 hours and additional amounts were administered subsequently when required with monitoring of the arterial acid base status The infusion was given continuously at the rate of 70 ml/kg/day by a No 5 French polyvinyl catheter in the umbilical vein it was maintained for at least 4 days and it was stopped only when no major therapeutic problems were considered to be present Heparinized plasma was given within 2 hours of collection from the donor Supplemental oxygen was administered to patients with cyanosis respiratory distress or apnoeic spells, and according to arterial oxygen studies when available Apnoeic episodes were treated only by bag and mask ventilation In the first part of the investigation only incubators with manual regulation of ambient temperature were used In the last part of the trial incubators with servoregulation of temperature became available providing 12 treated and 11 control subjects with a skin temperature of 36 –36 5 C Patients with bradycardia associated with 2 1 atrio ventricular block (9) were treated with 1% $CaCl_2$ intravenously

The gestational age was calculated from the first day of the last menstrual period and rounded to the nearest week Clinical observations included among others repeated recording of Silverman s score respiratory and heart rate and skin colour In many patients a detailed neurological examination was repeatedly performed by one of us (S P B)

The systemic systolic blood pressure (SBP) was measured indirectly from the radial artery by a modified xylol/pulse indicator instrument (7) The difference was also calculated between observed value and normal average value predicted from body weight gestational age and post natal age (Δ SBP mm Hg = observed – predicted SBP) according to a multiple regression equation obtained in another study (10)

Antero-posterior chest films were taken in duplicate on admission by a portable X ray machine (focal distance 90 cm exposure time 0 04–0 06 sec intensity 50–55 kV) without removing the infant from the incubator and were repeated when required Retrospectively, the chest X ray findings were classified as 1) markedly abnormal (i.e with severe and diffuse hypotransparency and/or fine granularity coarse nodularity, large patchy opacity) 2) without marked abnormality (i e absence of findings listed under 1).

Acid-base and oxygen determinations were performed on arterial (radial) or arterialized capillary blood by means of a micro-Astrup apparatus and a Clark type microelectrode. Carbon dioxide tension (P_{CO_2}) oxygen tension (P_{O_2}) and pH were corrected for the temperature of the infant. Additional details on methods of sampling measurement and calculation have been described elsewhere (8, 33) Other laboratory determinations and ECG tracings were also obtained, and have been reported elsewhere (11).

After microscopic examination of the lungs, the findings of interstitial and/or alveolar polymorphonuclear infiltration and of hemorrhage were graded from 0 to 3

Differences in average values were analysed by the Student s t test Differences in the mortality rate or in the prevalence of various situations were analysed by the χ^2 method Differences in survival curves were analysed by the Wilkoxon two sample test (rank sum test) assigning ranks to the survival time in dead infants and assuming a normal distribution for survivors (15)

RESULTS

The trial was performed from December 1965 to July 1968

During this period 17 babies admitted within 24 hours of birth and weighing 750–1 250 g were not included for the following reasons. died immediately after admission before completion of laboratory studies (5) acid-base studies not available because of failure of laboratory equipment (7) congenital malformation (2) stratified subgroups (see previous paragraph) already completed (3) 12 out of these 17 patients (71%) died within the first 10 days of birth

Findings on admission, and comparability of experimental groups

Some characteristics of treated and control babies have been reported in Table 2 and some clinical and laboratory findings in Table 3 The differences between the experimental groups were small and statistically not significant

Fluid and feeding actually administered

In treated babies the infusion was started on the average at 10 hours of age (in 15 in-

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[1] Regression equation for SBP of healthy low weight newborns (age 3–96 hours)
SBP mm Hg = 23 2 + 8 13 bw + 0 503 g a + 0 226 pn a – 0 0016 (pn a)^2
where bw = body weight (kg) g a = gestational age (weeks) and pn a = post natal age (hours)

Table 6 *Neonatal mortality in the whole series and in treated or control subjects according to some findings on admission*

	All subjects			Treated		Controls		p
	No of cases	% died	p	No of cases	died	No of cases	% died	treated vs. contr
Birthweight, kg								
≥1 04	40	45%	*	21	48	19	42	
<1 04	40	77		19	84	21	71	
Gestat. age weeks								
≥28	35	37	**	19	53	16	19%	*
<28	28	75		13	77	15	73%	
Sex								
males	38	63%		17	65	21	62	
females	42	60%		23	65%	19	53%	
Resp rate/min								
≤54	41	76	**	22	68	19	84	
>54	39	45		18	61%	21	33	
Chest X ray findings								
no marked abnormal	57	54		27	56	30	47	
marked abnormal	23	76%		13	85	10	70	
Arterial pH								
>7 323	39	44	**	15	47	24	42	
≤7 323	41	78		25	76	16	81	

Significance of difference in mortality rate * $p<0.05$ ** $p<0.02$ ** $p<0.01$

Apnoeic spells and neurological examination

Comparison was made between the number of treated and of control infants with apnoeic spells in the first 3 days and from 4 to 10 days of birth (separated also into those with 1 or 2 and those with more spells) Spells occurring within 2 hours of death were not considered No significant differences were found

In some of the infants a thorough neurological examination was performed at random by one observer (S P B) Out of the various findings elicited only the occurrence of tremors and cloni and the pattern of muscular tonus in the 2nd–3rd day of life were selected for presentation (When different findings were observed in the 2nd and in the 3rd day only observations in the 2nd day were considered) 23 treated and 26 controls were studied The incidence of tremors was very similar in the two groups The incidence of cloni and of increased muscular tonus was slightly higher in controls (cloni 9% in treated and 19% in control subjects increased tonus never in treated and 15% in controls) but the difference was not statistically significant Seizures were never observed

Heart rate and systolic blood pressure

Persistent bradycardia (i e heart rate below 85/min) not associated with apnoea or with agonal conditions was found in the 2nd day of life in 6 control patients This was always associated with 2 1 atrio-ventricular block, hyperkalemia and hypocalcemia and was cured by variable amounts of 1% CaCl intravenously These patients have been more extensively considered in another report (11)

In the 63 subjects with known gestational age 198 SBP determinations were performed in the first 4 days of life and the Δ SBP values were calculated The mean Δ SBP in treated and control babies at various age intervals (1st, 2nd 3rd and 4th day of birth) was compared, and no significant difference was found

Fresh human plasma administration and hemorrhage at post mortem examination

According to the plan of the trial (Table 1) 3 ml/kg of fresh human plasma were to be administered to infants in the treatment group as soon as possible after admission and to be repeated in the 3rd–4th day and possibly also

Table 4 *Mean amount of fluid and calories (oral+i v) actually administered to treated and control babies in the first 8 days of life (Amounts given in the day of death have not been considered)*

Day of life	Fluid ml Treated		Fluid ml Controls		Calories Treated		Calories Controls	
1	41	223	1	80	17	98	0 4	40 4
2	82		28		38		14	
3	100		51		43		26	
4	119	257	90	209	63	141	46	117
5	138		119		78		71	
6	139	446	130	446	87	292	85	297
7	145		145		97		97	
8	162		171		108		115	

the reported differences was statistically significant The most marked (but not significant $p=0.07$) discrepancy was represented by a higher incidence of intracranial hemorrhage in treated infants (48 %) than in controls (23 %) No central thrombosis or hepatic lesions were observed on gross post mortem examination However a very detailed examination of abdominal vessels together with microscopic studies of the liver had not been planned in advance and were rarely performed

Table 5 *Outcome and post mortem findings in the experimental groups*

	Treated	Controls
Number of cases	40	40
No ( ) died	26 (65 %)	23 (57)
Median age of death hours	81	42
No of autopsies	25	22
No ( ) c intracranial hemorrhage	12 (48 %)	5 (23)
No ( ) c visceral[a] hemorrhage	0	1 (5)[d]
No ( ) c other findings	0	2 (9)[e]
No of lung microscopic examinations	21	21
No ( ) c HMD	5 (24)	6 (29)
No ( ) c pneumonia[b]	2 (9)	1 (5)
No ( ) c hemorrhage[c]	4 (19 %)	4 (19)

[a] Pulmonary hemorrhage not included
[b] Grade 2 & 3 polymorphonuclear infiltration
[c] Grade 3 interstitial and/or alveolar hemorrhage
[d] Intestinal
[e] 1 c left kidney & ovary agenesis and 1 c moderate ileal stenosis

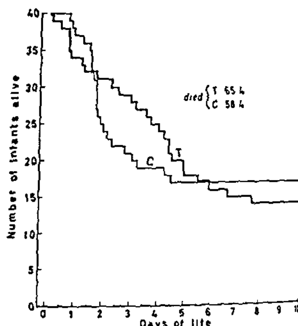


Fig 1 Neonatal survival curve in treated (*T*) and control (C) infants in the trial

Prognostic value of some findings on admission, and therapy

The possible effect of treatment on the mortality rate was also investigated after separating the infants according to some findings on admission The latter included above or below median birthweight gestational age respiratory rate and arterial pH sex, absence or presence of marked abnormality on chest film

In Table 6 the mortality rate in such categories of subjects has been reported When considering all infants (irrespectively of treatment), a significantly lower mortality was found in subjects with higher birthweight, gestational age respiratory rate and arterial pH whereas no significant differences were observed between males and females, or between infants with or without marked chest film abnormality When comparing treated and control infants within the same category the only significant difference ($p<0.05$) was found in the category with gestational age ≥ 28 weeks, with a lower mortality rate in control subjects However, in the overall context of the present results it is difficult to assign a definite meaning to this observation

compared to controls needs some comment It has been suggested that intracranial hemorrhage may result from osmolal load (25) However in the present study the difference in the incidence of intracranial hemorrhage between the experimental groups did not attain statistical significance In addition, in a controlled trial on babies with HMD and birthweight of 1 250–2 500 g performed in this Centre (37) a similar incidence of intracranial hemorrhage was found in babies with oral feeding or with i v infusion of glucose and $NaHCO_3$ Thus the conclusion seems warranted that none of the therapeutic regimens tested influenced *per se* neonatal mortality

The role of intravenous infusion with glucose and alkali in the therapy of newborns with very low weight

In newborns with the respiratory distress syndrom a decrease in mortality rate after alkali and glucose infusion has been observed by many workers (21–23 40) However Usher (43) reported improved survival in patients with birthweight above 1 5 kg, not in those with birthweight of 1–1 5 kg. In this Centre a controlled trial was performed on babies weighing 1 25–2 50 kg and an improved neonatal survival curve was found after infusion (37)

In newborns with birthweight of 750–1 500 g (with and without respiratory disease) Cornblath et al (14) found no significant mortality changes after i v infusion with glucose (and without $NaHCO_3$) as compared to oral feeding however when analysing retrospectively the results in the group with birthweight of 750–1 250 g, a significant decrease in mortality after i v infusion was found.

The present trial has provided conclusive evidence that, under our experimental conditions, no detectable improvement of neonatal survival could be obtained by i.v infusion therapy in subjects with very low birthweight and with or without pulmonary disease or early acidosis. Similar results were obtained in a controlled trial previously performed in this Centre on 40 infants with the same weight (31) In that trial the plan of oral or i v fluid administration was the same as in the present investigation with the exception that infants in the treatment group were given the infusion only if the arterial pH was below 7 30 in the early days of birth

However in previous studies on newborns with low weight hypoglycemia metabolic acidosis increased levels of NPN K and P and increased urinary output of Na K, and N were frequently observed these changes were enhanced by fasting, hypothermia and respiratory distress and were prevented or corrected at least in part by early oral feeding or infusion therapy (2 4 5 8 13, 16 19 24 28–30, 32 34, 35 38 39 41 42 45 46) In some investigations prevention of hyperbilirubinemia by early fluid administration has been reported (20 26 39 45 46) Some of these findings have been substantiated by laboratory observations performed in the course of the present trial (11) Since many of these abnormalities are potentially dangerous to the central nervous system the final evaluation of the role of intravenous infusion with glucose and alkali in newborns with very low weight must await long term follow up studies in survivors Thus it is suggested that, before such studies are available this therapy should be given routinely to newborns with very low weight The problem of duration of the infusion has been discussed elsewhere in greater detail (11)

Antimicrobial therapy mortality and pneumonia

In previous studies there was some suggestion but no conclusive proof that the administration of methicillin plus colistin (36) or of gamma globulin (1) was beneficial to low weight newborns In the present trial there was no difference in mortality rate after the two antimicrobial regimens tested (Table 1) and the incidence of pneumonia at post mortem examination was low both in the treated (9%) and in the control (5%) series Bacteriological studies were not performed routinely and therefore could not be used in order to evaluate

Table 7 *Incidence of significant hemorrhage upon post mortem examination in patients with or without plasma therapy*

	Autopsy			Lung microscopic examination	
	Total no	No ( ) of intracranial hemorrhage	No ( ) of other hemorrhage[a]	Total no	No ( ) of hemorrhage[c]
Subjects with plasma therapy	20	10 (50)	0 (0)	17	3 (18)
Subjects without plasma therapy	27	7 (26)	1 (4)[b]	25	5 (20)

[a] Not including pulmonary h
[b] Intestinal
[c] Grade 3 pulm hemorrhage

in the 5th–6th day of life However, because of practical difficulties, this plan was not followed in every case In fact, 6 infants (4 living less than 24 hours, and 2 living for 2 days) never received plasma 17 babies (7 living less than 72 hours, 6 living for 4–5 days, and 4 surviving) received only 1 administration only 17 subjects received 2 or 3 administrations

Thus, the 47 patients in this series with post mortem studies were divided into two groups the 20 infants in the treatment group who did actually receive at least 1 plasma administration and the 27 infants (including 5 babies in the treatment group) who never received plasma Post-mortem studies have been reported in Table 7 The incidence of intracranial hemorrhage was markedly but not significantly higher in subjects who received plasma (50%) as compared with those without (26%) plasma therapy The incidence of hemorrhage in other organs was fairly similar in the two groups

Major observations after the neonatal period

Retrolental fibroplasia was never observed on the routine fundoscopic examination performed before discharge Two infants belonging to the control group developed the pulmonary syndrome of Wilson Mikity and died at 2–3 months of age The remaining 29 babies were discharged from the Unit in the 3rd–4th month of life in apparently good health

DISCUSSION

Methodology

In the present trial a satisfactory comparability of the experimental groups was achieved by stratifying subjects on enrollment according to two prognostic parameters (i e weight and arterial pH) Luckily enough, these parameters proved retrospectively to be among the best discriminants with respect to neonatal mortality as compared with other prognostic factors considered in Table 6 The need for a careful selection of parameters when stratifying for experimental groups in controlled trials is apparent.

The treatment schedule differed from the control regimen by three respects (oral feeding vs i v infusion, antimicrobial regimen, fresh human plasma administration) This could have made the interpretation of results difficult if a difference in neonatal survival had been found between experimental groups However no such difference was observed In addition there was no evidence of beneficial effects of either antimicrobial regimens tested or of plasma administration when considering post mortem finding nor was there any evidence of possible adverse effects of either antimicrobial regimens umbilical vein catheterization or intravenous therapy With respect to the latter statement the finding of a markedly higher incidence of intracranial hemorrhage at post mortem examination in treated babies as

vented or corrected at least in part, by the early infusion with glucose and $NaHCO_3$. Since some of these abnormalities are potentially dangerous to the central nervous system the final evaluation of this therapy must await long-term follow up studies in survivors. It is therefore suggested that before such studies are available the early i.v. infusion with glucose and $NaHCO_3$ should be given routinely to newborns with very low weight.

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the effects of treatment The present evidence, although not conclusive, suggests that in our experimental conditions no difference in mortality due to pneumonia can be expected after either therapy

It may be of some interest to report that the present antimicrobial trial was performed because in a previous series (31) of autopsies in newborns with similar weight given kanamycin sulf (20 mg/kg/day for 5 days), the incidence of pneumonia was 26% This observation clearly shows that the population admitted to a given unit may change considerably in character with time, and that policies of antibiotics administration need to be continuously reevaluated The authors fully agree that antibiotics should be administered to low-weight newborns only when infection is definitely suspected However, they feel that in their working conditions routine antibiotic administration for a few days was justified, since often the previous history of their referred patients was unreliable or long in gathering

Fresh human plasma administration mortality, and hemorrhage

In the present investigation mortality and the incidence of hemorrhage at post mortem examination were similar in infants who did or did not receive fresh human plasma (3 ml/kg in the first day of life sometimes repeated in the 3rd and 5th day) On the other hand Gray et al (17) reported that the administration of 10 ml/kg of fresh human plasma to low weight newborns with clotting defects (as assessed by Thrombotest) lessened significantly the risk of death with intracranial hemorrhage Further investigation is clearly necessary including the serial evaluation of single coagulation factors before and after therapy

SUMMARY

A controlled therapeutic trial was performed in newborns with birthweight of 750–1 250 g 40 infants (the treatment group) received an i v infusion with glucose and $NaHCO_3$ from the 1st to the 4th–7th day of life, and increasing amounts of human milk from the 2nd day of life They also received, during the newborn period, 3 antibiotics (sodium penicillin G 500 000 U + methicillin 100 mg + colistin sulf. 8 mg per kg/day), a gamma globulin preparation (0 5 ml/day for 3 days) and fresh human plasma (3 ml every 2nd day in the first 5 days of birth) 40 infants (the control group) received only oral feeding with 10% glucose and human milk, starting in the 2nd day of life, and i m kanamycin (20 mg/kg/day for 5 days)

No difference between treated and control babies was observed with respect to the following findings neonatal mortality rate and survival curve on the whole series, neonatal mortality rate in babies with birthweight above or below 1 04 kg, with gestational age below 28 weeks, with above or below median respiratory rate or arterial pH on admission, with or without marked abnormality on chest film and in males or females (but, in subjects with gestational age above 27 weeks, a significantly lower mortality rate was found in controls), post mortem findings, incidence of apnoeic spells tremors and cloni, and pattern of muscular tonus Bradycardia associated with 2 1 atrioventricular block was observed in 6 controls in the 2nd day of birth No adverse effects presumably due to the therapeutic procedures so contrasted were demonstrated

It was concluded that in the present series of newborns with very low weight (*a*) the massive anti infectious therapy was not superior to the administration of a single broadspectrum antibiotic (*b*) the administration of fresh human plasma was not effective in preventing hemorrhage (but further evaluation is needed), (*c*) the routine i v infusion with glucose and $NaHCO_3$ did not improve neonatal survival as compared with oral feeding

However previous studies, as well as observations on the present series reported elsewhere (11) have shown that several biochemical abnormalities commonly seen in orally fed newborns with very low weight can be pre

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No difference between treated and control babies was observed with respect to the following findings neonatal mortality rate and survival curve on the whole series neonatal mortality rate in babies with birthweight above or below 1 04 kg with gestational age below 28 weeks with above or below median respiratory rate or arterial pH on admission with or without marked abnormality on chest film and in males or females (but in subjects with gestational age above 27 weeks a significantly lower mortality rate was found in controls) post mortem findings, incidence of apnoeic spells tremors and cloni and pattern of muscular tonus Bradycardia associated with 2 1 atrioventricular block was observed in 6 controls in the 2nd day of birth No adverse effects presumably due to the therapeutic procedures so contrasted were demonstrated

It was concluded that in the present series of newborns with very low weight (*a*) the massive anti infectious therapy was not superior to the administration of a single broadspectrum antibiotic (*b*) the administration of fresh human plasma was not effective in preventing hemorrhage (but further evaluation is needed) (*c*) the routine i v infusion with glucose and $NaHCO_3$ did not improve neonatal survival as compared with oral feeding

However previous studies, as well as observations on the present series reported elsewhere (11) have shown that several biochemical abnormalities commonly seen in orally fed newborns with very low weight can be pre-

the effects of treatment The present evidence, although not conclusive, suggests that in our experimental conditions no difference in mortality due to pneumonia can be expected after either therapy

It may be of some interest to report that the present antimicrobial trial was performed because in a previous series (31) of autopsies in newborns with similar weight given kanamycin sulf (20 mg/kg/day for 5 days), the incidence of pneumonia was 26% This observation clearly shows that the population admitted to a given unit may change considerably in character with time, and that policies of antibiotics administration need to be continuously reevaluated The authors fully agree that antibiotics should be administered to low-weight newborns only when infection is definitely suspected However, they feel that in their working conditions routine antibiotic administration for a few days was justified, since often the previous history of their referred patients was unreliable or long in gathering

Fresh human plasma administration mortality and hemorrhage

In the present investigation, mortality and the incidence of hemorrhage at post mortem examination were similar in infants who did or did not receive fresh human plasma (3 ml/kg in the first day of life sometimes repeated in the 3rd and 5th day) On the other hand Gray et al (17) reported that the administration of 10 ml/kg of fresh human plasma to low weight newborns with clotting defects (as assessed by Thrombotest) lessened significantly the risk of death with intracranial hemorrhage Further investigation is clearly necessary including the serial evaluation of single coagulation factors before and after therapy

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However previous studies as well as observations on the present series reported elsewhere (11), have shown that several biochemical abnormalities commonly seen in orally fed newborns with very low weight can be pre

vented or corrected at least in part by the early infusion with glucose and $NaHCO_3$. Since some of these abnormalities are potentially dangerous to the central nervous system the final evaluation of this therapy must await long term follow up studies in survivors. It is therefore suggested that before such studies are available the early i.v. infusion with glucose and $NaHCO_3$ should be given routinely to newborns with very low weight.

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A CONTROLLED TRIAL ON THERAPY FOR NEWBORNS WEIGHING 750–1 250 g

II Blood Chemistry and Electrocardiographic Observations in the Newborn Period

G BUCCI M MENDICINI A SCALAMANDRÈ L ANNIBALDI P G SAVIGNONI and S NODARI

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In fasting newborns with low weight hypoglycemia (3 38 43) metabolic acidosis (6 21) increased serum levels of NPN K and P increased urinary output of Na K and N (1 3 10 29 35 48 49) and hyperbilirubinemia (26) have been frequently observed These abnormalities were reportedly prevented or corrected at least in part by the early administration of oral feeding or of intravenous infusion with glucose and with or without $NaHCO_3$ (1 17 19 24 28 39 45 48 49 52 56) Early hypocalcemia was a common finding (5 14 47) and as suggested by a recent study it was possibly enhanced by the administration of $NaHCO_3$ (47)

In fasting newborns with very low weight metabolic acidosis (6) and signs of increased tissue catabolism (1 35 48 49) were even more frequent and severe and were associated with electrocardiographic abnormalities (7 31 33 48) However there are only limited prospective studies on blood chemistry and ECG abnormalities comparing oral feeding with intravenous infusion in large and well controlled groups of very small newborns As to the effect on neonatal mortality published results were inconclusive In fact, in one trial there was some suggestion but no conclusive proof of a decrease in mortality after intravenous infusion (11) whereas no changes in neonatal survival were reported in another study (50) In spite of these uncertainties routine early intravenous infusion has been advocated for all newborns with very low weight (2) and is currently applied in many Centres

Thus we performed a prospective trial on the effect of intravenous infusion with glucose and $NaHCO_3$ as compared with oral feeding in newborns weighing 750–1 250 g For reasons given elsewhere (32) two different antimicrobial regimens and the administration of fresh human plasma were also evaluated In this paper blood chemistry and electrocardiographic observations in the neonatal period will be presented Clinical findings and mortality data in the newborn period have been given in another report (32) Follow up studies are also in progress

MATERIAL AND METHODS

The material was the same as described in the previous report (32) The plan of treatment (summarized in Table 1 in the previous communication (32)) calculation of gestational age and classification of X ray findings were the same as reported previously (32)

Acid–base and oxygen determinations were performed on arterial (radial) or arterialized" capillary blood by means of a micro Astrup apparatus and a Clark type micro-electrode Carbon dioxide tension

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fants (for technical difficulties some electrolytes were not studied in part of the cases) Patients were selected at random but very sick subjects were usually not studied The results of blood studies some findings on admission and some data on therapy have been reported on Table 2 Upon admission treated patients had marked abnormality on chest film more seldom than the controls but their mean blood pH was lower

In the 2nd day of life the mean serum Na and Ca were identical in the two groups whereas the mean serum K and P and the BUN were significantly ($p<0.01$) higher in the controls Individual data have been illustrated in Fig. 4 from which it appears among other findings that serum K exceeded 8 mEq/l in 13 out of 22 controls (59 %) and only in 3 out of 18 treated patients (16.7%) The arterial Ht did not change appreciably from 1st to

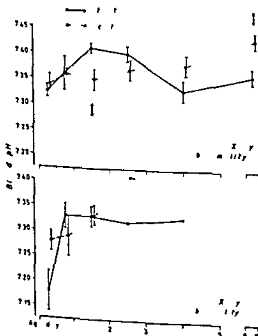


Fig 1 Mean ±S E of arterial pH in treated and control infants with or without severe abnormality on chest film on admission. Mean values from less than 3 observations, and S E from less than 4 observations, were not included Arrows indicate significant differences between means $p<0.05$ $p<0.01$

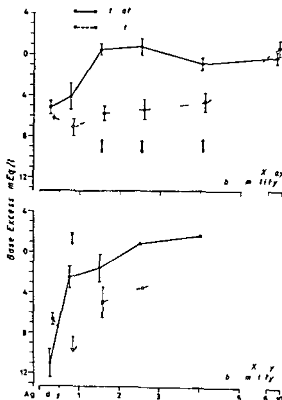


Fig 2 Mean ±S E of blood Base Excess in treated and control infants Other explanations as in Fig 1

2nd day of life in the controls and decreased moderately in treated babies

The correlation between various blood chemistry findings was investigated after pooling results in the treated and the control group Highly significant correlations were found between serum K serum P and BUN (Fig 5 *a b* and *c*) There was a weak but still significant correlation between blood pH in the 2nd day of life on the one hand and serum K or serum P on the other whereas no correlation at all could be demonstrated between blood pH in the 2nd day and BUN (Fig 6 *a b* and *c*) On the contrary the blood pH on admission did not bear any correlation with either serum K serum P or BUN The relationships between the various blood chemistry findings were apparently similar in treated and control infants in fact whenever a significant relationship was found and the regression line

Table 1 *Some characteristics and some laboratory findings on admission (mean ± S D or %) in infants in the trial*

	Treated	Controls
Number of cases	40	40
No ( ) c birthweight ≤1 kg	18 (45)	17 (42%)
No ( ) c gest age ≤28 weeks	22 (66)[b]	19 (61)[c]
No (%) c weight ≤10th perc for gest age[a]	5 (16%)[b]	3 (10 %)[c]
Age on admission hours	6 8±5 5	6 5±4 9
No ( ) c marked abnormality on chest film	13 (32)	10 (25)
Arterial acid-base status on admission		
pH	7 311±0 140	7 329±0 090
Base Excess mEq/l	−7 2±5 5	−7 2±4 0
P_{CO_2} mmHg	44 2±17 9	37 7±14 2

[a] According to the single birth intrauterine weight chart of Lubchenco et al (25)
[b] Known in 32 infants
[c] Known in 31 infants

(P_{CO}) oxygen tension (P_{O_2}) and pH were corrected for the temperature of the baby Additional details on methods of sampling measurement and calculation have been described elsewhere (6 37)

In some of the infants on the second day of life several blood components (serum K Na Ca P and BUN) and the ECG were studied simultaneously (i e within a time lapse not longer than 2 hours) In order to avoid hemolysis blood samples were taken in a plastic syringe allowed to clot into a test tube under mineral oil and centrifuged at slow speed Serum Na and K were measured by flame spectrophotometry serum Ca by the method of Kramer & Tisdall (22) in the first part and by the method of Mc Pherson (30) in the last part of the investigation (results by both methods were found to be reasonably reproducible) serum P by the method of Fiske & Subba Row (13) the blood urea nitrogen (BUN) by the method of Fawcett & Scott (12) The ECG was taken with a direct writing machine recording the usual leads (I II III aVR aVL aVF VR V V V V_6) Low voltage was frequently found and in such case a standardization of 1 mV = 20 mm was used

The total serum bilirubin was determined daily (and more frequently in the presence of rapidly increasing or dangerous bilirubin levels) starting from the time when a significant jaundice was clinically evident and until a definite decrease of serum bilirubin was observed The method of Malloy & Evelyn (27) was used Direct reacting bilirubin was determined at least once in subjects with total serum bilirubin higher than 17 mg/100 ml The hematocrit was determined on arterial or arterialized capillary blood after centrifugation in capillary tubes at 4 000 r p m for 5 min

Additional details on plans and methods of the investigation have been reported elsewhere (32)

RESULTS

Findings on admission and comparability of experimental groups

The trial was performed from December 1965 to July 1968 Some characteristics and some findings on admission in the treatment and in the control group have been reported in Table 1 The difference between the experimental groups were small and statistically not significant Additional information on clinical and laboratory findings on admission and on the amount of fluid actually administered has been given in another report (32)

Post-natal changes of acid-base parameters

In the first 10 days of life 257 pH Base Excess (BE) and P_{CO} determinations were performed on arterial (97%) or arterialized capillary (3%) blood In addition 20 BE determinations were performed on capillary or venous blood On this data, the post natal changes of acid-base parameters in treated and control infants were investigated However in view of the heterogeneity of clinical and pathophysiological conditions encountered in the present series, it was considered appropriate to analyse the data separately in subjects with or without marked abnormality on chest film

Results have been shown in Figs 1 to 3 On the average acidemia was present in the early hours of birth more markedly in babies with severe X ray abnormality In the first 2 days of birth the acid-base parameters showed minor changes in the controls whereas in treated infants the Base Deficit decreased and the pH increased After the 3rd day of life the pH was on the average lower in treated infants because of markedly higher P_{CO} levels

Serum electrolytes and ECG findings in the 2nd day of life

During the 2nd day of life blood chemistry studies (serum Na K, Ca and P BUN) were performed in 19 treated and 23 control in

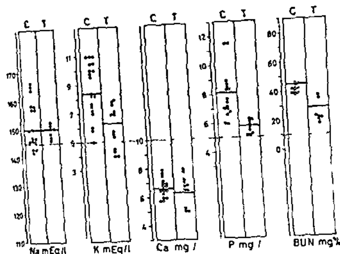


Fig 4 Blood chemistry findings in the 2nd day of life in treated (*T*) and control (*C*) infants considered in Table 2 Horizontal bars represent mean values and the dotted line the mean normal level

infants who survived for at least 5 days (in no case direct bilirubin exceeded 10% of the total) Thus only the latter group of infants was considered

Treated and control subjects were closely comparable with respect to mean birthweight gestational age incidence of RDS in the first two days of birth and incidence of ABO incompatibility with the mother s blood Treated infants received about twice as much fluid as the controls in the first 3 days of life and about 20 more in the 4th–5th day The incidence of hyperbilirubinemia (both ≥ 17 mg/100 ml and ≥ 20 mg 100 ml) was remarkably but not significantly ($p = 0\,06$ and $p > 0\,10$ respectively) higher in the control group There was a trend for hyperbilirubinemia to occur earlier in control than in treated infants in fact when considering control infants hyperbilirubinemia was first found in 4 case in the 4th day and in 3 cases in the 5th–6th day whereas in treated infants it was first discovered in the 4th day in one and in the 6th day in 2 cases In the treatment group the highest total serum bilirubin observed was of 20 2 mg/100 ml and the infant recovered spontaneously Four controls had peak total serum bilirubin between 22 and 26 mg/100 ml and received one or two exchange transfusions In 17 treated and 13 control infants determinations of arterial hematocrit both in the 1st and in the 4th–5th

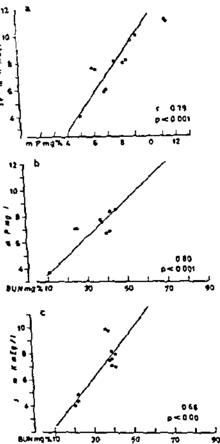


Fig 5 Correlation between serum K serum P and BUN levels in the 2nd day of life in treated (●) and control (○) infants considered in *Table 2* The correlation coefficient *r* the regression line and the statistical significance of *correlation* are also shown

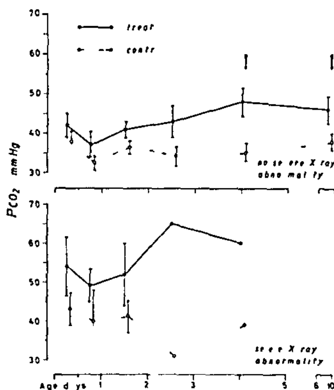


Fig 3 Mean ±S E of arterial Pco in treated and control infants Other explanations as in Fig 1

was calculated roughly the same proportion of values from the two groups was located above or below the line No correlation what soever was observed between serum Na or serum Ca on the one hand and the remaining blood findings in the first or second day of life on the other

In part of the infants with blood chemistry studies the ECG was simultaneously taken (Table 3) showing in about one third of the controls prolonged PR interval QT interval and QRS complex A QT interval longer than 0 40 sec was found in 7 controls and was associated in 6 instances with bradycardia due to 2 1 atrio-ventricular block (7) With the exception of one infant with a PR interval of 0 16 sec such findings were never observed in the 16 treated patients with ECG studies

The ECG abnormalities were always asso ciated with high serum K values in particular all subjects with QT interval longer than 0 40 sec had serum K higher than 9 5 mEq/l Af ter pooling the results from treated and control patients a significant correlation wa calculated between the serum K level and the duration of the QT interval (Fig 7) On the contrary, no correlation was present between the serum Ca level and the duration of the ECG parameters considered

The six controls with 2 1 atrio ventricular block received variable amounts of 1% $CaCl_2$ intravenously, and all of them recovered

Hyperbilirubinemia (Table 4)

Hyperbilirubinemia (i e peak total serum bili rubin ≥ 17 mg/100 ml) was never observed in the 31 infants (12 treated and 19 controls) who lived less than 72 hours, nor in the 12 infants (8 treated and 4 controls) who died in the 4th or 5th day of birth On the other hand hyperbilirubinemia was found in 10 of the 37

Table 2 *Data on admission and findings in the 2nd day of life (mean ± S D or %) in part of treated and control infants in the trial (Numbers in parentheses indicate the number of observa tions when different from the total number)*

	Treated	Controls
Number of cases	19	23
Data on admission		
Weight kg	1 05	1 10
Gestational age weeks	28 4 (14)	27 8 (20)
No ( ) c̄ marked X ray abnormality	1 (5)	4 (17)
arterial pH	7 31	7 35
Blood studies on 2nd day		
Serum Na mEq/l	149 7±12 4 (16)	150 2±8 8 (2?)
Serum K[a] mEq/l	6 3±1 8 (18)	8 4±2 0 (2?)
Serum Ca mg/100 ml	6 4±1 3 (16)	6 6±0 8 (?1)
Serum P[a] mg/100 ml	5 8±1 2 (14)	8 1±2 0 (19)
BUN[a] mg/100 ml	29 2±15 2 (15)	44 6±14 9 (15)
Arterial Ht difference (1st–2nd day)[a]	5 8±8 3 (16)	0 4±5 3 (15)
Therapy		
No ( ) c̄ servo contr of body temp	8 (42)	8 (35)
Mean amount of fluid (ml) given before blood studies	56[b]	13 5[c]

[a] Significance of difference between treated and controls $p<0\ 01$
[b] Only i v oral feeding never given
[c] Only oral 10 gluc milk given to one baby only

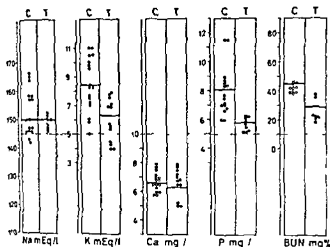


Fig 4 Blood chemistry findings in the 2nd day of life in treated (*T*) and control (*C*) infants considered in Table 2 Horizontal bars represent mean values and the dotted line the mean normal level

infants who survived for at least 5 days (in no case direct bilirubin exceeded 10% of the total) Thus only the latter group of infants was consid red

Treated and control subjects were closely comparable with respect to mean birthweight gestational age incidence of RDS in the fir t two days of birth and incidence of ABO in compatibility with th mother s blood Treated infants received about twice as much fluid as th controls in the first 3 days of life and about 20% more in the 4th–5th day The incidence of hyperbilirubinemia (both ≥ 17 mg/100 ml and > 20 mg/100 ml) was remarkably but not significantly ($p = 0\ 06$ and $p > 0\ 10$ respectively) higher in the control group There was a trend for hyperbilirubinemia to occur earlier in control than in treated infants in fact when considering control infants hyperbilirubinemia was first found in 4 case in the 4th day and in 3 cases in the 5th–6th day whereas in treated infants it was first discovered in the 4 h day in one and in th 6th day in 2 cases In th treatment group the high st total serum bilirubin observed was of 20 2 mg/100 ml and th i fant recovered spontaneously Four controls had peak total serum bilirubin between 22 and 26 mg/100 ml and received one or two exchange transfusions In 17 treated and 13 control infants determinations of arterial hematocrit both in the 1st and in the 4th–5th

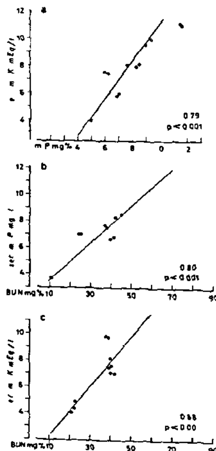


Fig 5 Correlation between serum K, serum P and BUN levels in the 2nd day of life in treated (●) and control (O) infants considered in Table 2 The correlation coefficient *r* the regression line and the statistical significance of correlation are also shown.

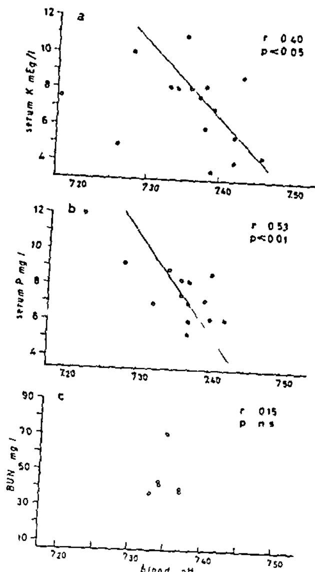


Fig 6 Correlation of arterial pH with serum K serum P and BUN (all results in the 2nd day of life) Other explinations as in Fig 5

Table 3 *ECG findings in the 2nd day of life in part of babies considered in Table 2*

	Treated	Controis
Number of cases	16	21
No ( ) c PR int >0 12 sec	1 (6)	6 (32)[c]
No ( ) c QRS int >0 04 sec[a]	0 (0)	8 (38)
No ( ) c QT int >0 40 sec[b]	0 (0)	7 (33)
No ( ) c 2 1 atrioventricular block[b]	0 (0)	6 (29)

[a b] Significance of difference between treated and controls [a] $p<0.01$ [b] $p<0.02$
[c] On 19 tracings data could not be calculated on 2 tracings with 2 1 atrioventricular block

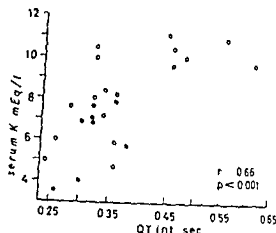


Fig 7 Correlation between serum K and QT interval in the 2nd day of life Other explanations as in Fig 5

day of life were available On the average, the Ht drop was only slightly greater in treated than in control infants

DISCUSSION

Interpretation of results

In the present study the management of treated and control infants differed by three respects oral feeding or i v infusion anti

Table 4 *Some findings (mean or %) relevant to the problem of hyperbilirubinemia in treated and control babies who lived for at least 120 hours*

	Treated	Controls
No of cases	20	17
Birthweight kg	1 10	1 11
Gestational age weeks	28 3[b]	29 1[c]
No ( ) c RDS[a]	11 (55)	7 (41
Fluid (i v and/or oral) given		
days 1–3	220	102
days 4–5	259	214
No ( ) c AB0 incompatibility	3 (15)	3 (18
No ( ) c peak total serum bilirubin		
≥17 mg/100 ml	3 (15)	7 (41)
>20 mg/100 ml	1 (5)	4 (24)
No ( ) c exchange transfusion	0 (0)	4 (24)
Arterial Ht difference (1st–4th/5th day)	8 1[d]	4 8

[a] RDS = Silverman s score >2 and/or resp rate >60/min for at least 48 hours after birth
[b] On 17 infants
[c] On 16 infants
[d] 17 observations
[e] 13 observations

microbial regimen administration of fresh human plasma However there is no reason to suspect that the different antimicrobial regimens or the plasma administration could affect the biochemical or cardiac findings reported in the present paper Therefore in the discussion dif ferent results in the experimental groups will be considered as related to oral feeding or to i.v infusion with water glucose and $NaHCO_3$

Metabolic effects of fasting and early i v infusion

In agreement with previous studies on infants with comparable weight post natal age and therapy (3 35 49) the present investigation showed conclusively that fasting newborns with very low weight often develop shortly after birth very high levels of serum K and P and of BUN which can be prevented at least in part by early infusion therapy Hyperkalemia and different serum K levels between infants with early infusion or routine oral feeding were not observed in another trial in the 4th day of life (11) In order to explain such dis crepancy the different ages of patien s studied should b taken into consideration as well as the different risk of hypoxia and hypothermia prior to admission in extramurally or intra mu ally born infants

Increased tissue catabolism and decreased renal function due in turn to prema urity fast ing perinatal stress and asphyxia have been postulated to explain these findings and have b en substantiated by balance studies in fast ing and early fed newborns (1 3 29 35 53 55) Th present data do not bring direct evidence on the etiology of these abnormalities but emphasize the role of extreme prematurity and of starvation In addition the close cor relation between changes of serum K serum P and BUN (Fig. 7) suggests that these abnor malities share to a great extent the same origin The weak (but still *significant*) inverse correla tion of blood pH with serum K and P (not with BUN) suggests that an additional mechan ism related to acidosis plays a role in the origin of hyperkalemia and hyperphosphatemia (but not of hyperazotemia) The regression lines calculated from the present data showed a decrease in serum K of 4 7 mEq/l and in serum P of 5 0 mg/100 ml, per unit pH in crease These values were somehow interme- diate between the coefficients of 3 and of 6 found experimentally in uncompensated acido- sis (respectively respiratory and non respira tory) in the dog (44) Among the mechanisms involved in these changes the release of intra cellular K in exchange with H (10) and splitting of organic phosphate compounds due to a fall in intracellular pH (16) must be con- sidered

As to hyperbilirubinemia reports on the ef fect of early as compared to late fluid and calories administration on serum bilirubin lev- els have b en contradictory and prevention of severe hyperbilirubin mia has been observed by some authors (19 24 45 52 56) not by others (4 11 17 20 28) The present results were in agreement with the former point of view but additional investigation possibly tak ing into account the many factors involved in the etiology of hyperbilirubinemia of pre- maturity (26 36) is necessary It may be ques- tion d whether the decrease in the incidence of hyperbilirubinemia after i v infusion was due to a b neficial metabolic effect or to hemo- dilution In the present series hemodilution as indicated by the drop of h matocrit after the day of birth in treated babies as compared with controls was on the average fairly marked in the second day of life (Table 2) but rather limited in the 4th–5th day (Table 4) when marked hyp rbilirubinemia was first found to occur For this reason and in consideration of th large extravascular pool of bilirubin in hy- perbilirubinemic infants (51) it seems unlikely that hemodilution might have contributed sig nificantly to the decrease in the incidence of hyperbilirubinemia after i v infusion

Blood glucose determinations were not per formed in the present study As reported else where (32) the incidence of clinical signs usu ally associated with hypoglycemia (such as ap- noeic spells increased muscular tonus tremors

and cloni (38)), was not significantly different in the present series of babies with or without i v infusion, although cloni and increased tonus were moderatly more frequent in fasting subjects

Early neonatal hypocalcemia

It has been previously shown that in newborns with low weight serum Ca levels decrease in the early days of birth, and that this decrease is greater the lower the weight (5, 14 47) Therefore, the very low serum Ca values observed in the present series in the second day of life were not surprising The pathophysiology of early neonatal hypocalcemia is largely unknown (34) In a recent investigation (47) early neonatal hypocalcemia was found to be associated with birth asphyxia prematurity, respiratory distress increased serum P and decreased blood pH and serum protein in the early hours of birth and with $NaHCO_3$ administration However the cause–effect relationship of these findings with hypocalcemia was not clear The present investigation, performed on subjects with a very narrow birthweight range showed that serum Ca levels were not related to serum K and P, to BUN or to blood pH levels and were similar in infants with or without i v infusion This suggests that early neonatal hypocalcemia, although related to prematurity and possibly also to perinatal distress was due to mechanisms differing from those involved in the serum electrolyte changes previously discussed and also that it was not secondary to the other blood chemistry abnormalities considered in the previous paragraph In addition it was shown that the administration of $NaHCO_3$ was not a cause of early hypocalcemia The possible relationship of hypocalcemia with ECG abnormalities will be discussed below Other adverse effects possibly related to hypocalcemia were not apparent

Electrocardiographic abnormalities

ECG abnormalities similar to those encountered in the present study have been previously reported in newborns with very low weight, and have been attributed to hypocalcemia hyperkalemia, or a combination of the two (7, 9 15, 31, 33, 46, 48) The present data, as well as more extensive investigations from this Centre (31) indicated that such ECG changes were certainly related to hyperkalemia However their relationship to hypocalcemia was uncertain In fact, on the one hand the ECG abnormalities were always associated with hypocalcemia and could be reversed by the i v administration of Ca, on the other hand no correlation was found between degree of ECG abnormality and serum Ca levels Thus it was not clear whether the effect of Ca administration was due to the correction of hypocalcemia or if it was rather to be considered as a pharmacological effect related to Ca K interaction in myocardial excitability and contractility (8 18) Further studies including the determination of ionized Ca are needed to elucidate this problem

Acid base status

In a previous trial on infants with HMD and birthweight of 1 25–2 5 kg (41) patients given i v infusion with glucose and $NaHCO_3$ showed when compared to patients with oral feeding higher Base Excess and blood pH values in the first two days of birth and similar blood pH but higher P_{CO_2} levels thereafter In the present series of infants with low weight and with or without pulmonary disease a similar pattern was observed except that in treated infants the late hypercarbia was more marked so that the mean blood pH was lower than in the controls Although such late hypoventilation after glucose and $NaHCO_3$ administration was not associated with an increased incidence of apnoeic spells (32) this finding should be regarded with some concern and further trials contrasting relatively liberal or restricted policies of $NaHCO_3$ administration after the first 2 or 3 days of birth seem indicated

Indications and policy of early i v infusion with glucose and $NaHCO_3$ in newborns with very low weight

In another report on the present series of newborns with very low weight (32) no difference in neonatal survival after oral feeding or i v infusion was observed On the other hand the present report in keeping with studies discussed above has shown that early acidemia hyperkalemia hyp razotemia hyperbilirubinemia and ECG abnormalities were prevented or corrected at least in part, by the early i v infusion Other investigations have shown that hypoglycemia frequently occurs in fasting newborns with low weight and can be prevented by early oral or i v feeding (4 11 17 28 38 39 43 56) It seems reasonable to suspect that in newborns with optimal perinatal care (such as subjects immediately transferred from the delivery room to the Intensive Care Unit under well controlled conditions) the incidence of such abnormalities would be lower than in the present series of extramurally born patients However since many of these abnormalities are potentially dangerous to the central nervous system it is suggested that while waiting for a final evaluation by long term follow up studies in survivors *the early i v infusion therapy* should be given routinely to newborns with very low weight

Nevertheless unnecessary and potentially dangerous therapeutic procedures should be avoided The present observations have shown that correction of acidemia was achieved by the i.v infusion therapy in the first 2 days of birth but that subsequently marked hypoventilation occurred The beneficial effect on serum electrolytes and ECG paramet rs was already obtained after few hours of infusion The decrease in the incidence of hyperbilirubinemia was *already* apparent in the 4th day of birth It seems therefore that the full benefit of early i v infusion with glucose and $NaHCO_3$ was in most cases obtained within the first 2 or 3 days of therapy On the other hand central thrombosis and hepatic lesions after umbilical vein catheterization have been reported with increasing frequency (40 42 54) and it has been shown that the incidence of such lesions increases markedly after 48 hours of catheterization (23) It is therefore suggested that unless uncommon problems are present the i v infusion therapy should not be administered for more than 2 or 3 days possibly associated with a fast oral feeding schedule which has been shown to be feasible in newborns with very low weight (11 45)

SUMMARY AND CONCLUSIONS

A controlled therapeutic trial was performed in newborns with birthweight of 750–1 250 g 40 infants (the treatment group) received an i v infusion with glucose and $NaHCO_3$ from the 1st to the 4th–7th day of life and increasing amount of human milk from the 2nd day of life They also received in the newborn period 3 antibiotics (penicillin methicillin and colistin) gamma globulin and fresh human plasma 40 infants (the control group) received only oral feeding with 10% glucose and human milk starting in the 2nd day of life and i m kanamycin

As compared to controls in treated infants *acidemia decreased more rapidly in the first* two days of birth but hypercarbia developed after the 3rd day

Blood electrolytes and ECG studies were performed in part of the infants in the 2nd day of life Markedly increased serum K and P and BUN levels were observed in controls and significantly lower values in treated infants Severe ECG abnormalities were associated with hyperkalemia in controls and were never observed in treated infants Serum Ca levels were low and similar in the two groups Hyperbilirubinemia occurred more frequently in controls and exchange transfusion was performed only in this group of infants

Since many metabolic abnormalities commonly occurring in fasting newborns with very low weight are potentially dangerous to the central nervous system the conclusive evaluation of the i v infusion with glucose and

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Key words Newborn with very low weight, intra venous infusion blood electrolyte ECG serum bili rubin

$NaHCO_3$ must await long term follow up studies in survivors While waiting for this evidence, it is suggested that this therapy should be given routinely to newborns with very low weight The present observations also suggest that, if a reasonably fast oral feeding schedule is provided, in most circumstances the infusion therapy should not be continued after the 3rd day of birth

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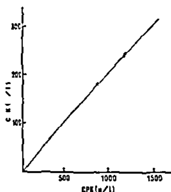


Fig 1 Comparison of two methods used for the determination of serum creatine phosphokinase Various sera were simultaneously analysed for CPK activity as mentioned under Methods The results obtained by the method of Okinaka et al (7) are plotted along the ordinate the results obtained by the method of Rosalki (11) along the abscissa

CoQ (hexahydrocoenzyme Q_4, 20 mg ml or 100 mg ml in corn oil) was supplied by one of us (K. F) In the case of the twins the control patient received commercial corn oil

CASE HISTORIES AND RESULTS

Case 1 and 2

K. H and K. S are identical male twins born May 27 1961 The family history was non-contributory They could sit unsupported when 8 months old and walked without support at the age of 16 months Walking difficulties soon became apparent By admission to our hospital (March 15 1968) at the age of 7 years they walked with a pronounced waddl and supported both hands on the knees when getting up from the prone position The muscles of the lower extremities and back were weak and the calves showed pseudohypertrophy The diagnosis of muscular dystrophy was confirmed by typical histological features in muscle biopsy and by typical findings on electromyography There were raised levels of several serum enzymes (GOT 63 and 99 U l GPT 60 and 10 U l LDH 850 and 1060 U l CPK 368 and 398 U l for K. H and K. S respectively)

CoQ treatment was started in May 1968 and initially the subjects were kept on a daily dose of 50 mg In September 1968 there was no change in the clinical picture therefore the daily dose was increased to 150 mg but without effect. Fig. 2 shows the results of a therapeutic study starting in March 1969 and lasting 4 months Before the experiment K. S and K. H were both on 150 CoQ daily (small dose) At this time both had markedly elevated activities of CPK During the period of treatment K. S received 1 g CoQ daily (large dose) while K. H received corn oil only There was a marked decrease of CPK in subject K. S during the period of treatment However there was an even more pronounced decrease in the control subject Fig 2 also shows the results of the aldolase determinations At the beginning of the experimental period both patients had elevated activities of serum aldolase with the higher activity in the control subject At the end of the experiment the control subject K. H showed an unchanged aldolase activity whereas there was a statistically insignificant increase in the patient receiving CoQ In July 1969 there was a definite decline in their motor power and they were no longer able to walk even with support CoQ treatment was discontinued in August 1969

Case 3

This boy (Ø S) was born March 11 1961 and was first admitted to our hospital on April 18 1967 The family history was non contributory He could sit without support when 7 months old and walked unsupported when he was 16 months but showed early difficulties in running and stairclimbing. On admis

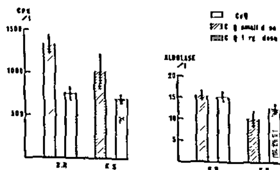


Fig 2 Creatine phosphokinase and aldolase activities in serum in a pair of identical twins Before the experiment both twins were given 150 mg CoQ daily (small dose") During an experimental period of 4 months subject K. S received 1 g CoQ daily (larg dose) while subject K H received corn oil only The enzyme activities were determined as described under Methods, the method of Rosalki (11) being used for CPK-determination Each bar represents the mean of 4-6 determinations and the standard error of the mean is indicated on the top of each bar

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COENZYME Q IN DUCHENNE MUSCULAR DYSTROPHY

A Preliminary Therapeutic Trial

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The muscular dystrophies which give rise to progressive weakness and wasting of skeletal muscles, represent a great challenge in current medical research Since no drug treatment is at present known which can change the course of the disease in any form of muscular dystrophy (18) it became of interest to study the effect of Coenzyme Q (CoQ) CoQ which is part of the electron transport system in mito chondria (9) exerts vitamin activity in the rab bit with nutritional muscular dystrophy (17 14) Intravenous administration of CoQ to these animals had a favourable therapeutic ef fect and was even lifesaving A less dramatic effect was observed in mice with a genetic type of dystrophy Thus Farley et al (2) observed physical improvement in genetically dystrophic mice after administration of CoQ Later Scholler et al (12) were able to demonstrate physical improvement as well as ex tension of life in these animals when given CoQ orally

In the present work we report a study on 4 boys with a Duchenne type of muscular dys trophy Two of these boys were identical twins and they offered an unusual opportunity for a controlled clinical experiment The patients were all followed during a period of more than 1 year Apart from a clinical evaluation we were in particular interested in the serum levels of creatine phosphokinase (CPK) and aldolase The serum levels of these as well as of s v eral other enzymes, are markedly elevat d in muscular dystrophy of the Duchenne typ (16)

METHODS

For the enzyme assays heparin blood was collected in the morning with the patient fasting and at rest The plasma was kept on ice until analysed CPK was initially determined as described by Okinaka et al (7) the phosphate produced being determined by the method of Fiske & SubbaRow (3) Reduced gluthathione was present in the incubation mixture Reagents produced by Sigma Chemical Company St Louis USA were used (Sigma Technical Bul letin No 661) The UV method of Tanzer & Gil varg (15) at 25 served as reference method and nor mal serum values for male adults are below 12 U/l and for female adults and children below 8 U/l Later CPK was determined at 25 by the method of Rosalki (11) using reagents produced by Calbiochem Luzern Switzerland Normal serum values are below 35 U/l and 20 U/l for male and female adults respectively with the mean value for children of 2 to 10 years of age slightly below that for female adults (19) There appears to be a linear relationship between the two methods (Fig 1) the CPK activities obtained by the method of Rosalki (11) being higher with a factor of 5 as compared with the method of Okinaka et al (7) Aldolase was determined by the UV method of Racker (8) at 25 using reagents produced by Boehringer Mannheim Germany Normal serum val ues for adults are below 6 U/l and for children below 3 U/l (4) The transaminases were assayed by the method of Reitman & Frankel (10) and lactic dehydrogenase (LDH) by the UV method of Wrob lewski & LaDue (20) at 25

ing itself in improved muscular strength (*b*) Reduced activities of CPK and aldolase which are serum enzymes with markedly increased activity in Duchenne muscular dystrophy

Concerning a clinical effect the therapeutic trial with the identical twins seems conclusive Thus both twins became clinically worse and stopped walking at the same time, in spite of the fact that one received 1 g CoQ daily whereas the other received corn oil only Of the 2 other patients studied one stopped walking while being treated like the twins whereas the other did not present any definite clinical change It appears therefore tha oral administration of this particular form of CoQ *does not affect the fundamental disturbance in* Duchenne muscular dystrophy

Before concluding that there is no deficiency of CoQ in Duchenne dystrophy two important questions should b answered 1 Does hexahydrocoenzyme Q_4 substitute for coenzyme Q_9 and Q_{10} which are the main forms of the vitamin biosynthesized in mammalian tissues? 2 Does orally administered CoQ reach its plac in the mitochondrial membranes of skeletal muscles?

It is of basic significance that hexahydrocoenzyme Q_4 has *in vitro activity in* the succinoxidase and the NADH oxidase systems Lenaz et al (6) found the activity of hexahydroco nzyme Q_4 to be comparable to that of coenzym Q_{10} in th succinoxidase system and slightly less than that of coenzyme Q_{10} in the NADH oxidase system

The vitamin activity of hexahydrocoenzyme Q_4 was first demonstrated in the nutritionally dystrophic rabbit where oral administration was lifesaving (14) By contrast the rabbit failed to recover after oral administration of coenzyme Q_{10}, which is the homologue normally present in rabbit tissue (1) These observations clearly demonstrated that to obtain a th rapeutic response *in vivo* transport requirements as well as structural requirements have to be met. Although hexahydrocoenzyme Q_4 appears to reach its enzyme site after oral administration in the rabbit, this might not be the case in humans And even if hexahydrocoenzyme Q_4 reached the skeletal muscle mitochondria it might not have the structural specificity necessary to restore a possible inadequate electron transport in Duchenne dystrophy

It is generally agreed that the activity of CPK in serum is elevated in most cases of Duchenne dystrophy and that this enzyme is a sensitive parameter for the activity of the disease Thus Goto et al (5) found the serum levels of CPK to be related not only to the age of the patient and the duration of the disease but also to the progression and the severity of the dystrophy It appears therefore that an improvement as well as a deterioration of Duchenne dystrophy might lead to only moderately elevated activities of CPK in serum

As shown in Table 1 a rather marked decrease of CPK was observed in the subjects Ø S and J H J during treatment with CoQ There was no corresponding clinical improvement On the contrary the clinical condition of Ø S deteriorated during this period whereas the disease was rather stationary in the case of J H J The plasma CPK of Ø S seemed to vary independently of the dose of CoQ (Fig 3) showing a net decrease during the period of treatment Also in the twins a decrease in CPK activity was observed This decrease which was associated with reduced physical ability was of a similar order of magnitude in the treated patient and the control It is therefore most likely that the decrease in plasma enzyme activities observed are coincidental and not caused by the CoQ treatment A possible exception is subject J H J whose clinical condition was rather stable during the test period and where the decrease in CPK and aldolase activities cannot be attributed to a change in the activity of the disease

Further clinical studies should be performed when other forms of the coenzyme are available The particular derivative of CoQ used in the present study was well tolerated and gave no side effects even at high doses

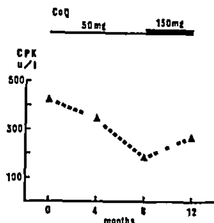


Fig 3 Creatine phosphokinase during 1 year with CoQ treatment Subject Ø S was given 50 mg CoQ daily for 8 months and then 150 mg daily for 4 months He was admitted to the hospital at 4 monthly intervals The points represent the mean of 5–12 determinations on separate days CPK was determined by the method of Okinaka et al (7)

sion he walked with a pronounced waddle and supported both hands on the knees when getting up from the prone position The muscles of his hips and shoulders were weak and there was pseudohypertrophy of his legs The diagnosis of muscular dystrophy was confirmed by electromyography and by examination of serum enzymes GPT showed an activity of 128 U/l GOT 91 U/l and LDH 740 U/l CPK was not examined at the first admission

CoQ treatment was started February 1968 Fig 3 shows the CPK levels during treatment with 50 mg and later 150 mg per day During the first 8 months his CPK dropped substantially from more than 400 U/l to about 200 U/l His clinical condition however remained unchanged After 8 months the daily dose was increased to 150 mg During another 4 months there was no improvement in the clinical condition and CPK increased to slightly less than 300 U/l When seen as an outpatient in July 1969 there was a marked decline in the motor power Thus from May 1969 he had not been able to walk CoQ was discontinued in August 1969

Case 4

This boy (J H J) was first seen June 1968 aged 11 years and 2 months The family history was noncontributory He could sit without support when he was 7 months, and walked at 12 months of age but fell quite easily and had difficulties in getting up from the prone position He was able to climb stairs, dress himself swim and ride a bicycle but continued to present muscular weakness At examination he walked with a waddle and with a slight drop-foot on both sides His skeletal muscles were generally weak with rather marked pareses of the hip muscles The electromyography pattern was in agreement with a myogenic affection Muscle biopsy showed atrophic muscle fibres GOT GPT and LDH were within normal limits whereas the CPK activity of serum was elevated (197 U/l)

Treatment with CoQ was started shortly after the first admission, and he was treated with increasing doses After an initial improvement in his muscular strength his condition remained rather stable and in October 1969 he could still climb the stairs

The activities of CPK and aldolase in serum of the patients Ø S and J H J kept on CoQ 1 g daily, are shown in Table 1 There was a marked decrease in the activities of both enzymes during a 4 month period In the case of Ø S the decrease is statistically significant for both enzymes

DISCUSSION

In the present work we were looking in particular for (*a*) A clinical effect of CoQ manifest

Table 1 *Creatine phosphokinase and aldolase activities in plasma during treatment with CoQ*

At the beginning of the test the CoQ dose was increased from 150 mg to 1 g per day After 4 months on this dose the subjects were readmitted for clinical and laboratory evaluation The enzyme activities were determined before and after the 4 month period

Subject	Creatine phosphokinase *u*/l plasma[a]		Aldolase *u*/l plasma[a]	
	Before	After	Before	After
Ø S	1 298 ± 45 (6)[b]	829 ± 87 (5)[b]	24 0 ± 4 0 (6)	11 4 ± 1 2 (4)[c]
J H J	872 ± 100 (5)	285	18 1 ± 4 9 (5)	8 9

[a] ±S E M [b] $p<0\ 001$ [c] $p<0\ 02$

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PERINATAL ASPHYXIA AND RESIDUAL PLACENTAL BLOOD VOLUME

NATALIE E FLOD and BRUCE D ACKERMAN

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The present study was undertaken to determine the effect of perinatal asphyxia on the distribution of blood volume between the fetus and placenta At present, it is unknown whether asphyxia results in pooling of blood in the placenta or transfer of placental blood to the fetus

MATERIAL AND METHODS

Thirty two vaginally delivered term infants were evaluated clinically by means of one minute Apgar score The infants had their umbilical cords clamped as quickly as possible at birth, and in all instances prior to the first breath A segment of cord was removed between two clamps for umbilical arterial blood sampling The arterial blood was drawn into a heparinized syringe which was then immediately placed in ice The blood was analyzed for pH using a Radiometer micro blood gas apparatus

An additional clamp was left on the umbilical cord and, after delivery of the placenta, it was dried quickly to remove maternal blood from its external surfaces. The placenta was then suspended in a funnel and the blood drained into a graduated cylinder as described by Redmond et al (6) The placenta was left in the funnel until spontaneous drainage ceased and, before removal, gentle pressure was applied over the maternal surface to express the last small quantity of placental blood The volume of blood which drained into the cylinder was expressed in ml per kg body weight of the infant Hematocrit was measured in cord venous blood and in capillary blood obtained from the baby s heel at 4 hours of age The heel was warmed for one minute prior to sampling

This work was supported by PHS Grant RRO 5351-08 National Institutes of Health Bethesda Maryland, USA

RESULTS

Apgar scores for the 32 infants ranged from 2 to 9 with a median of 7 The mean cord arterial blood pH was 7 25 with a range of 6 96 to 7 38 Seven infants had Apgar scores of 2 3 or 4 These 7 infants had a mean arterial pH 7 12 with a range of 6 96 to 7 27 Of these 7 3 severely asphyxiated infants (Apgar score 2–3) had pH values of 6 96–7 04 which were markedly lower than any other values in the group These data are illustrated in Fig 1 and agree with other studies of cord blood acid base balance (2) The mean residual placental blood volume (RPBV) was 32 ± 13 ml/kg body weight The 3 infants with the lowest cord arterial pH values had RPBV s which averaged 20 ml/kg body weight, which is approximately 1 S D below the group mean The relationship between cord arterial pH and RPBV is illustrated in Fig 2

The mean change in hematocrit from birth to 4 hours was +14% with a range of −4% to +30% Two of the 3 most severely asphyxiated infants (Apgar 2–3 and pH 6 96–7 04) had hematocrit changes of +30% and +23% which were markedly greater than the mean Only one other infant had an increase in hematocrit as large as that shown by these 2 infants The relationship between cord arterial pH and change in hematocrit over the first 4 hours is shown in Fig 3

SUMMARY

The effect of hexahydrocoenzyme Q_4 (CoQ) has been studied in 4 cases of Duchenne muscular dystrophy, including a pair of identical twins

CoQ was given by the oral route in doses increasing from 50 mg to 1 g daily In the case of the twins one received CoQ, whereas the other was given corn oil as a control

During a period of treatment of more than one year no improvement in muscular strength was observed On the contrary, there was a steady progression of the disease in 3 of the 4 cases with an identical clinical course in the twins

No overall consistent decrease in CPK or aldolase activities was observed during the period of treatment but there was a statistically significant decrease of CPK and aldolase for one boy

The absence of overall beneficial changes during this particular trial might have been because (a) *hexahydrocoenzyme Q_4 is intrinsically in*active in Duchenne dystrophy (b) the dose level was too low, (c) the disease in these particular boys was too advanced

Besides these limitations this trial also does not exclude the possibility that other homologs of the coenzyme Q group may have therapeutic benefit in human muscular dystrophy

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RESULTS

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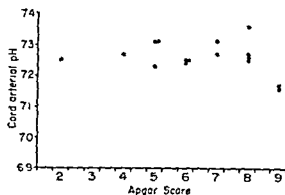


Fig 1 Apgar score at one minute of age and cord arterial blood pH

DISCUSSION

It has been reported that perinatal asphyxia results in arterial hypotension (4) This hypotension could, theoretically, be due to reduced blood volume Redmond et al (6) presented data indicating that placental transfusion does not occur if the cord is clamped prior to the first breath Thus hypotension might be expected in asphyxiated infants since early clamping is often carried out prior to the onset of respiration in order to facilitate resuscitation

There are no direct data concerning the effect of fetal asphyxia on fetal placental blood volume distribution although Dawes (1) showed that injection of epinephrine or norepinephrine in fetal lambs caused a decrease in fetal volume apparently due to a transfer of blood to the placenta

On the other hand, Yao et al (9) studied blood volume in the first hour of life in normal and asphyxiated infants delivered by Caesarean section The umbilical cords were clamped prior to extraction of the infant from the uterus They found a mean circulating blood volume of 66 ml/kg in 13 normal infants and 90 ml/kg in 5 asphyxiated infants One possible interpretation of these data was that asphyxia had caused a transfer of blood from placenta to fetus prior to delivery A possible explanation for such a redistribution would be the greater tendency for the umbilical artery, by comparison with the umbilical vein to constrict in response to asphyxial stimuli (3)

Other explanations are possible for the blood volume data reported by Yao et al, as the authors themselves noted (9) It is possible that asphyxia resulted in an expansion of total circulating volume, rather than transfer from the placenta Alternatively, asphyxia might have damaged capillary membranes and so allowed leakage of the labelled albumin into the extra vascular space resulting in false measurement

The present study is complimentary to the study of Yao et al (9), since we measured RPBV while they measured the infant's circulating volume Our results suggest that asphyxia does result in transfer of blood from placenta to fetus or, in other words that the larger circulating volumes observed by Yao et

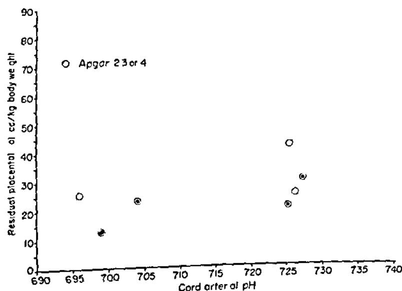


Fig 2 Cord arterial blood pH and RPBV Infants with low Apgar score and low pH tend to have small RPBV's

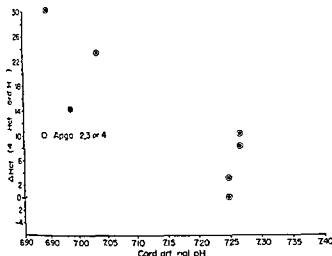


Fig 3 Cord arterial blood pH and increase in hematocrit from birth to 4 hours Infants with low Apgar score and low pH tend to have large increases in hematocrit

al in asphyxiated infants were in fact due to transfer from placenta to fetus We used cord arterial blood pH as a measure of perinatal asphyxia and related this observation to RPBV Yao et al (8) recently observed that total fetal placental circulating blood volume at birth has a mean value of 105 ml/kg body weight with a very small range of distribution Furthermore they demonstrated by clamping the cord at various intervals after birth, that as RPBV declines the infant s circulating volume increases by a like amount the sum of the 2 fractions remaining constant whether the cord is clamped immediately at birth or 3 min later Thus it is possible to measure RPBV and draw inferences from this measurement about the circulating blood volume of the infant

Our mean value for RPBV was 32 ml/kg body wt which is nearly identical with that reported by Yao et al (8) for infants with immediate clamping of the cord In our 3 most severely asphyxiated infants the RPBV values of 12 to 25 ml kg suggest by comparison with the data of Yao et al (8) that as much blood had already been transferred *in utero* as would ordinarily be transferred in the first 45 to 180 sec after delivery

Two of the 3 infants with severe asphyxia and low RPBV's also had large increases in hematocrit over the first 4 hours of life It has been observed that a large placental transfusion results in a large increase in hematocrit over the first few hours of life due to a rapid decline in plasma volume (7) Therefore our data on hematocrit at birth and at 4 hours tend to support the concept that RPBV s were in fact low because placental transfusion had already occurred *in utero* It is also possible however that impaired peripheral circulation was the cause for the higher capillary hematocrits in the more severely asphyxiated infants

It would appear from our data that hypotension following perinatal asphyxia probably results from factors other than hypovolemia

SUMMARY

The present data suggest that perinatal asphyxia may result in a transfer of blood *in utero* from placenta to fetus The data contain no suggestion that asphyxia causes pooling of fetal blood in the placenta

ADDENDUM

Philip et al (5) recently reported a study of RPBV in infants with early clamping of the umbilical cord Unexpectedly low RPBV s were observed in infants with clinical signs of fetal distress in labor or low Apgar score one minute after delivery Our data are in agree

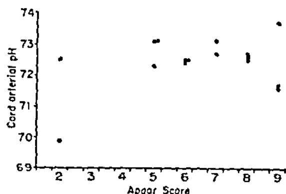


Fig 1 Apgar score at one minute of age and cord arterial blood pH

DISCUSSION

It has been reported that perinatal asphyxia results in arterial hypotension (4) This hypotension could, theoretically, be due to reduced blood volume Redmond et al (6) presented data indicating that placental transfusion does not occur if the cord is clamped prior to the first breath Thus hypotension might be expected in asphyxiated infants since early clamping is often carried out prior to the onset of respiration in order to facilitate resuscitation

There are no direct data concerning the effect of fetal asphyxia on fetal placental blood volume distribution although Dawes (1) showed that injection of epinephrine or nor epinephrine in fetal limbs caused a decrease in fetal volume apparently due to a transfer of blood to the placenta

On the other hand, Yao et al (9) studied blood volume in the first hour of life in normal and asphyxiated infants delivered by Caesarean section The umbilical cords were clamped prior to extraction of the infant from the uterus They found a mean circulating blood volume of 66 ml/kg in 13 normal infants and 90 ml/kg in 5 asphyxiated infants One possible interpretation of these data was that asphyxia had caused a transfer of blood from placenta to fetus prior to delivery A possible explanation for such a redistribution would be the greater tendency for the umbilical artery, by comparison with the umbilical vein to constrict in response to asphyxial stimuli (3)

Other explanations are possible for the blood volume data reported by Yao et al, as the authors themselves noted (9) It is possible that asphyxia resulted in an expansion of total circulating volume rather than transfer from the placenta Alternatively, asphyxia might have damaged capillary membranes and so allowed leakage of the labelled albumin into the extra vascular space resulting in false measurement

The present study is complimentary to the study of Yao et al (9) since we measured RPBV while they measured the infants circulating volume Our results suggest that asphyxia does result in transfer of blood from placenta to fetus or in other words, that the larger circulating volumes observed by Yao et

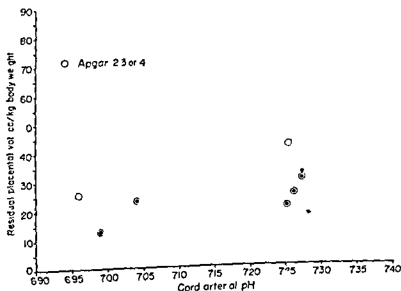


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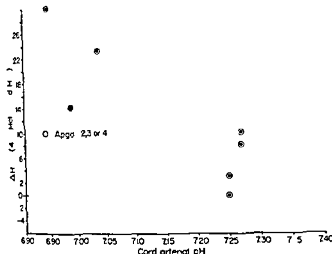


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URINARY EXCRETION OF FREE AND CONJUGATED GLUCURONIC ACID IN JAUNDICED NEWBORN

A GOTLIEB I NIR and J PESACH

From the Department of Paediatrics "A Tel Aviv University Medical School Government Hospital Zahalon" Jaffa and Department of Applied Pharmacology Hebrew University Jerusalem Israel

There is considerable variation in the intensity and duration of what has been called physiological jaundice in the newborn infant (5 10 13) It has been suggested by Matsuda & Shirahata (9) that lowered hepatic glucuronization as an expression of depressed enzymatic activity of the glucuronyl transferase system may play a role in many such examples of transient hyperbilirubinemia

We set out to explore this possible correlation (without having recourse to biopsy study) between varying degrees of neonatal jaundice and the level of impairment or immaturity of glucuronyl transferase function A significant parameter of the latter appeared to be expressed in the ratio between the urinary excretion of free and conjugated glucuronic acid

MATERIALS AND METHODS

Morning samples of urine were collected from newborn male infants for the first 4 days of life and then preserved at 5 C The urine was collected by attaching a small plastic bag to the penis and leaving it in situ for an hour or more until it contained sufficient urine for analysis All urine samples were analyzed for free and conjugated glucuronic acid

Serum bilirubin levels were simultaneously determined by the method of Malloy & Evelyn (8) on each of these 4 days and the infants accordingly classified into two groups "jaundiced" in whom the serum bilirubin level showed a continuing rise on the fourth day of life and "non jaundiced" in whom it had begun to decline by the fourth day (4) In all 62 infants were examined 37 showing jaundice and 25 being non jaundiced

In addition comparisons were made with the urinary excretion of free and conjugated glucuronic acid in the morning specimens of 13 premature infants and 15 healthy adult males None of the subjects under study received any drugs

Supported by grant from the Ministry of Health Jerusalem

Determination of glucuronic acid

The free and conjugated components of the total urinary glucuronic acids were determined by a modified version of the procedure described by Nir et al (12) Aliquots of urine (3–5 ml) were passed through a 9 × 100 mm Amberlite resin column The resin CG4B 200 mesh was saturated and regenerated three times until finally prepared in its formate form The columns containing the urine were washed with 100 ml distilled water which was discarded and then eluted with 100 ml aqueous 2 M NaCl

The total glucuronic acid in the eluate was measured with naphthoresorcinol before and after treatment with sodium borohydride (3) In this way the free and conjugated glucuronic acid contents of the urine were determined For each determination 5 ml of the eluate were treated with 1 ml of 0 4 M sodium borohydride and from this a 2 ml aliquot was used for the naphthoresorcinol reaction The naphthoresorcinol reagent was prepared according to Nir (11)

RESULTS

A significant difference ($p < 0\ 001$) in the ratio of free to conjugated glucuronic acid was found between the two groups In the infants with increasing jaundice the free acid constituted about 55% of the total urinary glucuronic acid compared to 42% in the non jaundiced group (Table 1)

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though also decreased in newborn without manifest jaundice this output and its ratio with free glucuronic acid approaches the values of normal adults It would appear that the physiological mechanism of conjugation is most depressed in premature and in physiologically jaundiced infants

The low rate of excretion of urinary glucuronides could be due to the still incompletely developed glucuronyl transferase located mainly in liver microsomes and found to be chiefly responsible for glucuronide synthesis (7 14) Such reduced function of the newborn liver would be reflected in both low glucuronization capacity and a higher serum bilirubin level A low urinary content of conjugated glucuronic acid is suggested as an indicator of this inadequacy of liver function in respect of the enzymatic activity of glucuronyl transferase

Our findings would appear to be in accord with those in animal experiments demonstrating that liver glucuronyl transferase is relatively inactive at the time of birth (1 2 7)

A reduced capacity to conjugate exogenous substances such as aminophenols and others has been defined in the newborn infant (15 16) The glucuronizing metabolism embracing these chemicals may not exploit the same pathways as that of endogenous substrates It has been reported for instance that glucuronization of chloramphenicol is not impaired in premature infants with higher than normal bilirubin levels (6) Our studies indicate that in newborn infants especially premature and jaundiced a low glucuronization capacity is a characteristic reflection of their physiological immaturity of liver function

Although a low urinary content of conjugated glucuronic acid fairly consistently coexisted with a high plasma bilirubin level jaundice was not always accompanied by decreased glucuronide excretion perhaps because the hyperbilirubinemia in some cases may predominantly derive from hemolytic element (4)

A high percentage of free urinary glucuronic acid would itself indicate depressed or pathological liver function at least in respect of its glucuronization capacity The excretion rate of both free and conjugated urinary glucuronic acid should provide diagnostic information to help distinguish the underlying type of jaundice Jaundice with a high free and low conjugated urinary glucuronic acid would indicate impaired liver function in this context Indeed several preliminary tests in obstructive and hepatocellular jaundice have shown a low rate of conjugated urinary glucuronic acid to occur only in the latter type an aspect requiring confirmation on a larger scale

SUMMARY

Free and conjugated components of the total urinary glucuronic acid were analyzed daily during the first 4 days of life in 62 newborn male infants The newborn were divided into two groups jaundiced 37 infants in whom the serum bilirubin level on the fourth day of life was either the same or higher than the level on the third day and "non jaundiced 25 infants in whom the serum bilirubin level showed a fall on the fourth day after an initial rise during the first 3 days The percentage of free glucuronic acid excreted was found significantly higher in the jaundiced infants

Premature infants studied for comparison demonstrated an even higher percentage of free glucuronic acid excreted in the urine than the jaundiced infants On the other hand in healthy adult males the percentage of free glucuronic acid excreted was found to be the lowest

No significant difference was found in the total glucuronic acid concentrations of the jaundiced and non jaundiced infants

It could be concluded therefore that when a high percentage of free glucuronic acid is excreted in the urine this probably indicates impaired conjugating capacity of the liver

Table 1 *The percentage of free glucuronic acid (free total ratio = F/T) in the urine of various groups*

Group	F/T ± S D of the mean ( )	Number in group
Premature (birth weight less than 2 kg)	66 6±9 8	13
Jaundiced newborn	55 1±6 4	37
Non jaundiced newborn	41 9±7 6	25
Adults	36 2±3 6	15

↕ = $p<0\ 001$ ⇕ = $p<0\ 01$

In the 13 premature infants run parallel for comparison 66% of the total urinary glucuronic acid proved to be free whilst only 36% did so in the 15 healthy adult males (Table 1)

The correlation between the percentage of free urinary glucuronic acid (F/T) and the serum bilirubin in the jaundiced and non jaundiced infants during their first 4 days of life is shown in Table 2

In order to analyze the relationship of the ratio of free to conjugated urinary glucuronic acid between the jaundiced and non jaundiced infants the number of infants with F/T more and less than 50% was calculated in each respective group (Table 3)

It can be seen that on the first day of life there is already a difference between the F/T ratios of the jaundiced and non jaundiced infants the disparity becoming highly significant ($p<0\ 001$) between second and fourth day of age, climaxing on the fourth day (Table 3)

The specific gravity of the urine samples was determined and no difference was found between the jaundiced and non jaundiced infants (1013–1016)

Table 3 *The percentage of free (F/T) urinary glucuronic acid of jaundiced (J) and non jaundiced (N J) newborn during the first four days of life*

	Day of life							
	1*		2**		3**		4**	
F/T	J	N J	J	N J	J	N J	J	N J
>50	20	7	25	4	30	6	[illegible]	2
<50	12	16	11	20	6	[illegible]	4	19
Total no newborn	32	23	36	24	36	18	36	[illegible]

* $p<0\ 05$ ** $p<0\ 001$

DISCUSSION

It is believed that the above results indicate that the measure of free and conjugated hexuronic acids of which glucuronic acid is the chief component, excreted in the urine is a relatively simple index of the efficiency of hepatic glucuronide synthesis in the newborn

The output of conjugated glucuronic acid was found to be lowest in a group of premature infants yet almost as low in most newborn infants with physiologic jaundice Al

Table 2 *Mean urinary glucuronic acid values (F/T) and serum bilirubin levels (S B) in jaundiced and non jaundiced newborn infants during the first four days of life*

Day of life	Non jaundiced (25 infants) Glucuronic acid: Free (mg/100 ml)	Conjugated (mg/100 ml)	Total (mg/100 ml)	F/T ( )	S B (mg/100 ml)	Jaundiced (37 infants) Glucuronic acid: Free (mg/100 ml)	Conjugated (mg/100 ml)	Total (mg/100 ml)	F/T ( )	S B (mg/100 ml)
1st	10 6	12 9	23 5	45 1	1 22	9 2	9 1	18 3	50 3	1 78
2nd	10 7	15 9	26 6	40 2	2 76	11 5	10 0	21 5	53 5	4 23
3rd	8 0	10 1	18 1	44 2	3 76	10 8	8 6	19 4	55 7	6 60
4th	5 5	7 8	13 3	41 4	2 97	9 0	6 2	15 2	59 2	8 25

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CHRONIC HYPOMAGNESEMIA WITH MAGNESIUM DEPENDENT HYPOCALCEMIA

I A New Syndrome with Intestinal Magnesium Malabsorption

S NORDIO A DONATH F MACAGNO and R GATTI

From the Department of Paediatrics G Gaslini University of Genoa Genoa Italy the Department of Paediatrics University of Berne Berne Switzerland and the Department of Child Health Paediatric Institute "Burlo Garofalo University of Trieste Trieste Italy

A new form of tetany and convulsions with chronic hypomagnesemia and secondary hypocalcemia has been discovered in recent years (1? 28 29 35 36 42)

The present study concerns another patient with this disease who was followed for 3 years[1]

The clinical course will be briefly described and the results of many metabolic investigations will be presented and discussed The results of these investigations were compared with those obtained in control children

To evaluate better the influence of Mg deficiency on Mg metabolism we also studied a control boy fed with a low Mg and normal-Ca diet

In a second paper the relationship between Mg and Ca P metabolism will be taken into consideration

CASE REPORT

A 7 months old italian boy was admitted to the hospital with convulsions and typical signs of tetany Irritability and psychomotor retardation were observed from the age of 4 months. Vitamin D and calcium therapy was previously prescribed at home without any effect

This investigation was supported by grant 4580 from Swiss National Foundation for Scientific Research (Fond National Suisse de la Recherche Scientifique)

The case was presented for the first time at the Annual Meeting of the European Society for Pediatric Research (Vienna, August 1968)

Plasma calcium phosphorus and alkaline phosphatase were respectively 7 5 mg/100 ml 8 mg/100 ml and 48 8 i u (normal values 17 to 100 i u)

Tetanic E M G hypocalcemic E C G tracings and convulsive potentials on E E G were demonstrated No X ray findings of rickets were observed Tibia and femur metaphysis appeared a little thickened

The course of the disease is summarized in Fig 1

No therapeutic effect was obtained with barbiturate intravenous Ca gluconate and oral CaCl administration (total dose of Ca^{++} 5 8 g during 8 days) At this time plasma magnesium was measured and was found to be low (0 8 mg/100 ml normal values between 1 9 and 2 5 mg/100 ml) A diagnosis of hypoparathyroidism with possible Mg-deficiency (13) was made but prolonged therapy with i v Ca gluconate (total dose of Ca^{+} 15 4 g) i m injection of Lilly" parathyroid extract (total dose 4130 i u) 10% MgSO solution (total dose of Mg 2 g) and vitamin D (total dose of 7 5 mg) was only partially successfull On this therapy plasma calcium remained at subnormal levels (on only one occasion it rose to 9 mg/100 ml) plasma phosphorus remained normal but on two occasions it rose to 9 and 7 7 mg/100 ml plasma magnesium never reached normal levels Convulsions became less frequent but both tetanic manifestations and irritability remained unchanged

Only a high oral intake of Mg glycerophosphate (3–7 g/day) completely normalized the clinical picture ECG EMG EEG plasma calcium and phosphorus Plasma magnesium rose (once up to 1 9 mg/100 ml) but practically never attained complete normal levels Each time Mg supplementation was discontinued irritability tetany (once convulsions) EEG ECG EMG alterations and later hypocalcemia and hyperphosphoremia reappeared (Fig 1) I Q at the age of 32 months was 68

Diarrhoea frequently occured both on normal diet and on Mg supplementation

The patients sister and parents have a normal plasma magnesium level

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I A New Syndrome with Intestinal Magnesium Malabsorption

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A new form of tetany and convulsions with chronic hypomagnesemia and secondary hypocalcemia has been discovered in recent years (17 28 29 35 36 42)

The present study concerns another patient with this disease who was followed for 3 years[1]

The clinical course will be briefly described and the results of many metabolic investigations will be presented and discussed The results of these investigations were compared with those obtained in control children

To evaluate better the influence of Mg deficiency on Mg metabolism we also studied a control boy fed with a low Mg and normal-Ca diet

In a second paper the relationship between Mg and Ca P metabolism will be taken into consideration

CASE REPORT

A 7 months old italian boy was admitted to the hospital with convulsions and typical signs of tetany Irritability and psychomotor retardation were observed from the age of 4 months Vitamin D and calcium therapy was previously prescribed at home without any effect

This investigation was supported by grant 4580 from Swiss National Foundation for Scientific Research (Fond National Suisse de la Recherche Scientifique)

The case was presented for the first time at the Annual Meeting of the European Society for Pediatric Research (Vienna August 1968)

Plasma calcium phosphorus and alkaline phosphatase were respectively 7 5 mg/100 ml 8 mg/100 ml and 48 8 i u (normal values 17 to 100 i u)

Tetanic E M G hypocalcemic E C G tracings and convulsive potentials on E E G were demonstrated. No X ray findings of rickets were observed Tibia and femur metaphysis appeared a little thickened

The course of the disease is summarized in Fig 1

No therapeutic effect was obtained with barbiturate intravenous Ca gluconate and oral CaCl administration (total dose of Ca 5 8 g during 8 days) At this time plasma magnesium was measured and was found to be low (0 8 mg 100 ml normal values between 1 9 and 2 5 mg/100 ml) A diagnosis of hypoparathyroidism with possible Mg-deficiency (13) was made but prolonged therapy with i v Ca gluconate (total dose of Ca 15 4 g) i m injection of Lilly" parathyroid extract (total dose 4130 i u) 10% MgSO solution (total dose of Mg^{+} 2 g) and vitamin D (total dose of 7 5 mg) was only partially successfull. On this therapy plasma calcium remained at subnormal levels (on only one occasion it rose to 9 mg/100 ml) plasma phosphorus remained normal but on two occasions it *rose to* 9 and 7 7 mg/100 ml plasma magnesium never reached normal levels Convulsions became less frequent but both tetanic manifestations and irritability remained unchanged

Only a high oral intake of Mg glycerophosphate (3–7 g/day) completely normalized the clinical picture E C G E M G E E G plasma calcium and phosphorus Plasma magnesium rose (once up to 1 9 mg 100 ml) but practically never attained complete normal levels Each time Mg-supplementation was *discontinued* irritability tetany (once convulsions) E E G E C G E M G alterations and later hypocalcemia and hyperphosphoremia reappeared (Fig 1) I Q at the age of 3 months was 68

Diarrhoea frequently occured both on normal diet and on Mg-supplementation

The patient s sister and parents have a normal plasma magnesium level

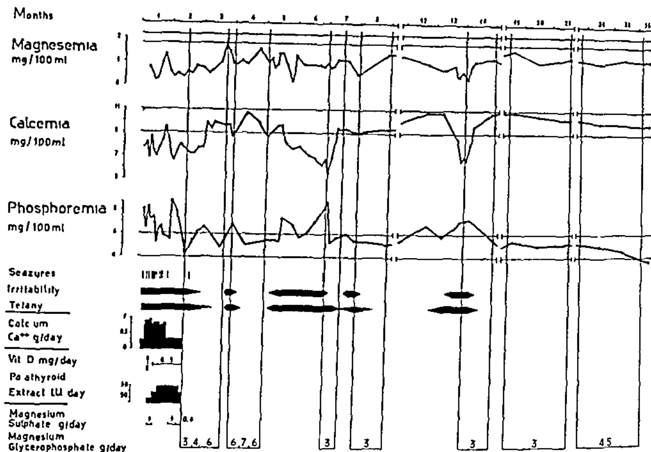


Fig 1 Clinical evolution and biochemical data of the case of primary hypomagnesemia on different treatments

METABOLIC INVESTIGATIONS

Methods Serum and urine Mg were determined by either the Mann & Yoes (21) or the atomic absorption spectrophotometric (Perkin & Elmer 303) (41) methods The latter was used for Mg determination in ashed feces food red cells cerebrospinal fluid (c s f) and sweat

^{28}Mg *total pool was determined according to* Raynaud & Kellershon (31) and Neer et al (26) ^{28}Mg intestinal absorption was determined by giving 20 μCi of this radioisotope by mouth without carrier after a 12 hours fast and by measuring the radioactivity of the feces of 5 days

The balance of stable Mg was performed in a metabolic ward *according to the method of* Rover (34) Body composition and exchangeable electrolytes were determined according to the method of Veall & Vetter (46)

Other investigations were performed by commonly accepted methods

RESULTS

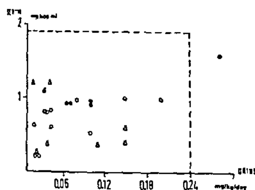
A low urinary elimination of *Mg* always corresponded to hypomagnesemia (Fig 2) Mg balance was variable but certainly reduced and on one occasion negative Intestinal absorption of both stable Mg and radioactive ^{28}Mg was also *reduced* (35) when compared to our control children on normal diet (*Table 1*)

Mg metabolism was also studied by i v injection of ^{28}Mg 19 days after discontinuing Mg therapy (plasma magnesium 1 1–1 2 mg/100 ml) Exchangeable pool accretion , urine and intestine excretion lied clearly lower than in the case studied by Salet et al and in normal adults (35) and likely *than in normal* children of the same age (Table 2)

Mg concentration in sweat was increased while it was within normal range in red cells and c s f (Table 1)

Intracellular K and K_e/Na_e ratio were low (Table 3)

With very low plasma magnesium the determination of some enzymes in serum (transaminase cholinesterase *glutamic dehydro*



ig ? Relationships between serum and urine Mg 1 the case of primary hypomagnesemia

:enase) red cells (glucose-6-P-dehydrogenase) and muscle (creatin phosphokinase) gave normal results

Glucagon i v injection (0 03 mg/kg) provoked an increase of blood glucose (130°) and blood lactate (60°)

In the intestinal mucosa Mg-dependent ATP ase histochemically studied (in the presence of Mg sulphate) appeared normal

Blood and urine electrolytes besides Mg Ca and P were not significatively modified

Table 2 *Mg metabolism of the patient with primary hypomagnesemia studied by* ^{28}*Mg i-v injection*

Exchangeable pool (mg/kg)	34 43
Accretio (mg/kg/day)	6 45
Urinary excretion (mg/kg/day)	0 019 (2 03 °)
Intestinal excretion (mg/kg/day)	0 141 (2 59)

Na Cl K Ca and P concentration in sweat was normal

Blood glucose plasma proteins lipides bilirubin and alpha amino nitrogen were normal as well as routine examination of c s f

No alteration of urinary 17 KS 17 KGS Porter Silber steroid was found Blood cortisol after ACTH stimulation was normal there were no signs of hyperaldosteronism

Fecal fat (one 5 days period) on normal diet was within normal range (0 6 g/day) xylose test (5 hours urinary elimination) intestinal mucosa (light microscopy) and intestinal X ray findings were normal.

An alteration of the apical part of the cytoplasm of epithelial cells of the intestinal mucosa was observed on electron microscopy dilated endoplasmic reticulum and mainly mitochondria swelling (Fig 3) The brush border was normal

Table 1 *Determinations of Mg in the patient and in control subjects on normal Mg diet or on low Mg diet*

	Mg balance								
	Intake (mg 5 days)	Output (mg 5 days) Stools	Output (mg 5 days) Urine	Balance (mg 5 days)	Intestinal absorption ( )	^{28}Mg Intestinal absorption (5 days)	C S F (mg/100 ml)	Sweat (mg/l)	Red cells (mg/g Hb)
Patient on normal diet	?77 ?0	31 75	0 57	5 1?	– 0 16				
	77 ?0	– 5 00	0 49	51 71	+ 18 83				
	77 ?0	195 70	0 75	+ 80 75	+ 29 13	+ 2..44	2.43	0 335	0 23
Mean values	77 ?0	34 15	0 60	+ 4..45	+ 15 93				
Control subjects on normal diet	857 50	?81.50	69 95	+ 506 05	+ 67 17				
Control subject on low Mg diet						+ 75 04	1 32-..76	0 025–0 130	0 18
						+ 56 07			
						+ 60 39			

Table 3 *Na_e and K_e body content of the patient with primary hypomagnesemia*

	Na_e (mEq/kg)	K_e (mEq/kg)
24 hour exchangeable pool	54 30 (norm 41 5)	21 80 (norm 40 0)
Extracellular	46 40	1 56
Residual (cell and bone Na_e)	7 90	
Intracellular		20 23

Renal function studies (creatinine clearance azotemia urine examination urine concentration power aminoaciduria glycosuria) gave normal results

In the control boy fed with low Mg diet (Mg intake of 2 3 mg/kg/day), plasma magnesium and urinary magnesium dropped from 2 to 1 2 mg/100 ml and from 52 to less than 10 mg/day respectively Intestinal absorption was clearly greater than in the patient with idiopathic hypomagnesemia (Table 1)

DISCUSSION

Nutritive requirement to maintain normal Mg homeostasis has been calculated to lie between 4 and 6 mg/kg/day in adults but it is certainly higher in growing children (18 37) Nevertheless it is exceedingly high in our patient (about 30–70 mg/kg/day)

In spite of this high intake plasma Mg never

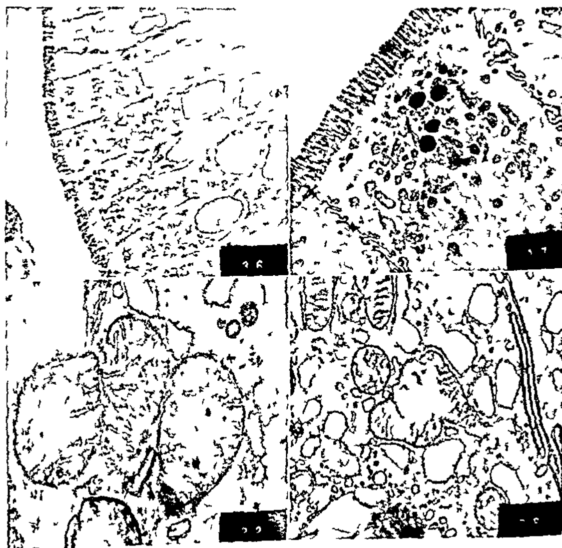


Fig 3 Intestinal mucosa of the patient with primary hypomagnesemia (electron microscopy)

attaines completely normal levels (Fig 1) when compared to normal values as determined by modern methods (4 5 21 43 45)

The exchangeable pool and the accretion of Mg are reduced more than in the case of Salet et al. (35)

In the case of Paunier et al (29) both Mg and K were reduced in muscle Because of a technical mistake we were not able to perform this determination but we found a diminished intracellular K and a low K_e/Na ratio (8 46) This agrees with the above mentioned results of Paunier et al (28) and with those obtained in Mg-deficient animals (23 47)

Mg-dependent ATP ase was normal in the intestine but the histochemical reaction was performed *in vitro* by adding Mg sulphate Therefore we cannot exclude an impairment of cation transport in chronic hypomagnesemia as a consequence of an *in vivo* reduction of membrane ATP ase regulating this transport secondary to Mg-depletion (20)

Erythrocyte Mg (Table 1) expressed in mg/1 is similar to that of Salet s case (58 mg/l) and corresponds to normal values reported by other authors (4 38 44)

When we consider our own and the previously published cases (28 29) we can draw the following *preliminary* conclusions on this disease

1 hypomagnesemia is associated to a body Mg-deficiency (exchangeable pool accretion muscle Mg)

2 Mg-deficiency is neither diffuse (normal c.s.f and red cell Mg) nor so important that it determines an impairment of the Mg dependent metabolic activities (normal glycolysis)

3 there may be a secondary alteration of cation transport in tissues.

On the basis of these observations the hypothesis of either an increased excretion or a reduced absorption of Mg can be postulated

Urinary magnesium directly depends on the supply and on the intestinal absorption of magnesium With Mg-deficiency when renal and endocrine functions are normal it diminishes to less than 12 mg/day in both adults (10 11 39) and children (our subject fed with low Mg diet) It is very low in our patient (Fig 2 and Table 3) and therefore it cannot explain hypomagnesemia

Intestinal secretion of Mg (1 35 40) was not increased (Table 3) Mg-concentration was increased in the sweat (Table 1) but this increase alone cannot adequately explain the Mg-deficiency in the patient

Therefore the impairment of Mg absorption from the intestine seems to be the most probable explanation

This hypothesis is also discussed by Friedman et al (12) and Paunier et al (29) but these authors studied only stable Mg balance Salet et al (35) who determined stable Mg balance on Mg supplement and ^{8}Mg absorption (40 %) excluded the defect of the intestinal Mg transport

In our case intestinal Mg malabsorption is clearly demonstrated by both stable Mg balance and oral ^{8}Mg administration (Table 1) Diarrhoea which as after experimental oral Mg load (7) frequently appeared further emphasizes this pathogenetic interpretation It is particulary interesting to draw attention to the difference in the ^{8}Mg absorption in the patient and in the control boy fed with low Mg diet although considering the plasma magnesium (0 52 mg/100 ml and 1 2 mg/100 ml respectively) Mg requirement ought to have been greater in the former than in the latter (Table 1)

Paunier et al (29) speculate whether in children a poor utilization of Mg in the intestine is sufficient to produce a Mg-deficiency and a rapid fall of plasma magnesium The results obtained in our control subject on low Mg diet allow us to answer Paunier s question positively

Mg is chiefly absorbed in the small intestine (3 6 17 30) This absorption depend on the intestinal transport function (14 16) and is influenced by foods such as proteins (22) phytate phosphate fatty acids (2) vita

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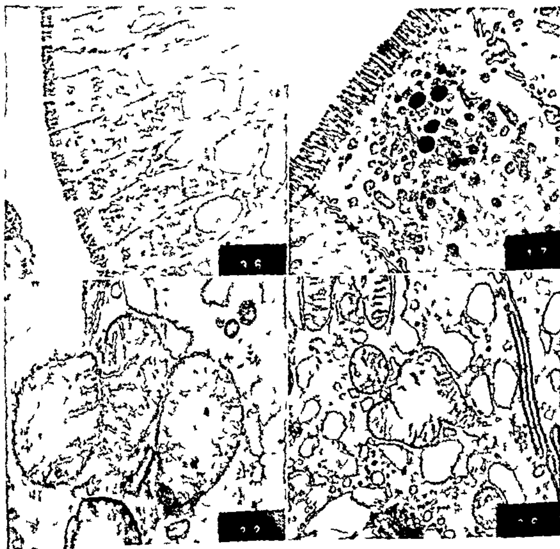


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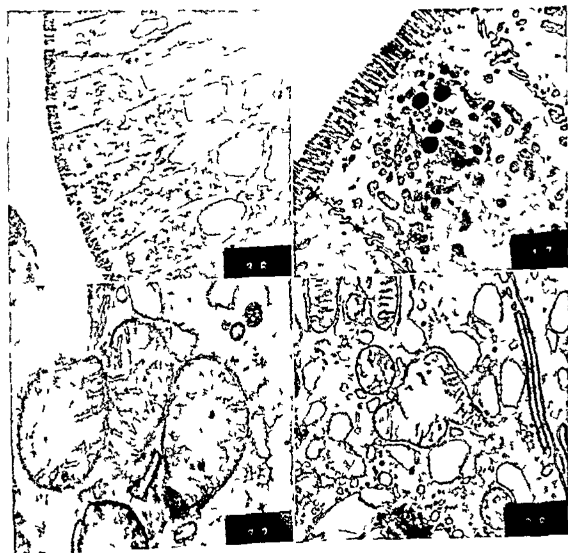


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min D (24 48) parathormone (19) and growth hormone (15)

In our patient no other manifestations of *intestinal malabsorption* were found The diet was not responsible for the hypomagnesemia and the patient did not have rickets Parathyroid function will be discussed in the next paper but there are no cases of hypoparathyroidism in which such a malabsorption has been demonstrated No other endocrine disorders (hyperaldosteronism) are evident

In conclusion '*chronic idiopathic hypomagnesemia is associated to a primary Mg-malabsorption* This interpretation agrees with the opinion formulated by Seip[1] and more recently by Strømme et al (42)

For the present no definitive pathogenetic interpretation is possible for this new intestinal disease

ATP ase transport system is not primitively impaired in the intestinal mucosa The swelling of mitochondria of epithelial cells (Fig 3) is neither constant (42) nor a specific finding (27) in Mg malabsorption although a relationship between Ca and Mg transport, vitamin D and parathormone mitochondria volume and function has been already emphasized on the basis of experimental investigations (9, 25, 27, 32 33)

Considering that the intestine is able to transport sufficient Mg only when its concentration in the intestinal lumen is unusually high the hypothesis of an impairment of the simple diffusion of Mg seems to be rather acceptable (42)

CONCLUSIONS

The new syndrome of chronic hypomagnesemia with Mg dependent hypocalcemia is characterized by primary Mg malabsorption An alteration of the simple diffusion of Mg seems likely It may be associated with mitochondria swelling

[1] Our results concerning Mg malabsorption were presented at the Symposium on Inborn Errors of Metabolism (Zurich June 1968) on the discussion of Seips communication

Taking into consideration the high concentration of Mg in sweat, we demonstrated for the first time a more extensive impairment of Mg transport cannot be excluded in this condition

Decreased urinary excretion of magnesium was present but we did not study the renal clearance of this cation Therefore we cannot establish the actual tubular reabsorptive function in this disease Considering possible functional relationships between intestinal mucosa and tubule we think it will be interesting to study the transport of Mg in other cases of Mg malabsorption

SUMMARY

Description of a case of chronic hypomagnesemia with secondary hypocalcemia tetany and convulsions followed for a period of 3 years from the age of 7 month Primary malabsorption of magnesium has been demonstrated Mitochondria of intestinal mucosa cells were found to be swollen Mg-concentration was increased in sweat

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ATP ase transport system is not primitively impaired in the intestinal mucosa The swell ing of mitochondria of epithelial cells (Fig 3) is neither constant (42) nor a specific find ing (27) in Mg malabsorption although a rela tionship between Ca and Mg transport, vita min D and parathormone mitochondria vol ume and function has been already emphasized on the basis of experimental investigations (9 25 27, 32 33)

Considering that the intestine is able to transport sufficient Mg only when its con *centration in the intestinal lumen is unusually* high, the hypothesis of an impairment of the simple diffusion of Mg seems to be rather acceptable (42)

CONCLUSIONS

The new syndrome of chronic hypomagnese mia with Mg dependent hypocalcemia is characterized by primary Mg malabsorption An alteration of the simple diffusion of Mg seems likely It may be associated with mito chondria swelling

[1] Our results concerning Mg malabsorption were pre sented at the Symposium on Inborn Errors of Met abolism (Zurich June 1968) on the discussion of Seip s communication

Taking into consideration the high concen tration of Mg in sweat we demonstrated for *the first time a more extensive impairment of* Mg transport cannot be excluded in this con dition

Decreased urinary excretion of magnesium was present but we did not study the renal clearance of this cation Therefore we cannot establish the actual tubular reabsorptive func tion in this disease Considering possible func tional relationships between intestinal mucosa and tubule, we think it will be interesting to study the transport of Mg in other cases of Mg malabsorption

SUMMARY

Description of a case of chronic hypomag nesemia with secondary hypocalcemia, te tany and convulsions followed for a period of 3 years from the age of 7 month Pri mary malabsorption of magnesium has been demonstrated Mitochondria of intestinal mu cosa cells were found to be swollen Mg-con centration was increased in sweat

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min D (24 48), parathormone (19) and growth hormone (15)

In our patient no other manifestations of intestinal malabsorption were found The diet was not responsible for the hypomagnesemia and the patient did not have rickets Para thyroid function will be discussed in the next paper but there are no cases of hypopara thyroidism in which such a malabsorption has been demonstrated No other endocrine dis orders (hyperaldosteronism) are evident

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II *A Study of the Relationship between Magnesium Calcium and Strontium*

S NORDIO A DONATH F MACAGNO and R. GATTI

From the Department of Paediatrics G Gaslini University of Genoa Genoa Italy the Department of Paediatrics University of Berne Berne Switzerland and the Department of Child Health Paediatric Institute Burlo Garofalo University of Trieste Trieste Italy

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n this paper we shall attempt to evaluate complex relationship between Mg Ca and on the basis of investigations performed the case of idiopathic hypomagnesemia a itrol boy fed with a low Mg diet and a control children who received a Mg supment in the di t

METHODS

balance of stable Ca was determined according Royer (30)

Sr and ⁷Ca intestinal absorption was determined respectively giving 10 μCi and 3 μCi of these ioisotopes by mouth without carrier after a 12 rs fast and by measuring the radioactivity of the s of 5 days Both isotopes are separated by gam spectrometry

Ca total pool was determined according to Ray d & Kellershon (28) and Neer et al (22)

Other determinations were performed by com nly acceptable methods

Parathyroid extract (Lilly) activity was established studying renal clearance of P in control children

The low Mg diet administered to a control boy contained 2 3 mg/kg day Ca content of the diet was normal

Other control children were given a normal diet containing a supplement of 5 g/m /day of Mg glycephosphate

RESULTS

Ca P and Sr metabolism in idiopathic hypomagnesemia

The dependence of normal calcium and phos phorus blood levels on Mg supplements and the resistance to parathyroid extract and vita min D treatment were demonstrated in our patient (Fig 1 of the previous paper)

Hypocalcemia was always associated with low urinary calcium values (0 09–0 7 mg/kg/day) On more or less prolonged Mg therapy with plasma calcium between 7 and 10 mg/100 ml urinary calcium fluctuated between 0 3 and 2 2 mg/kg/day

Calcium balance was positive and calcium intestinal absorption was only a little lower than in control subject studied under the same conditions (Table 1) ⁸ Sr intestinal absorption was on three occasions practically zero either on normal diet or on Mg supplement (Table 1) The ⁴⁷Ca i v injection performed after Mg therapy was discontinued and when plasma calcium was very low (6 6 mg/100 ml) and plasma phosphorus increased (7 1 mg/100 ml)

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In this paper we shall attempt to evaluate the complex relationship between Mg Ca and Sr on the basis of investigations performed in the case of idiopathic hypomagnesemia a control boy fed with a low Mg diet and a few control children who received a Mg supplement in the di t

METHODS

The balance of stable Ca was determined according to Royer (30)

^{85}Sr and ^{47}Ca intestinal absorption was determined by respectively giving 10 μCi and 3 μCi of these radioisotopes by mouth without carrier after a 12 hours fast and by measuring the radioactivity of the feces of 5 days Both isotopes are separated by gammaspectrometry

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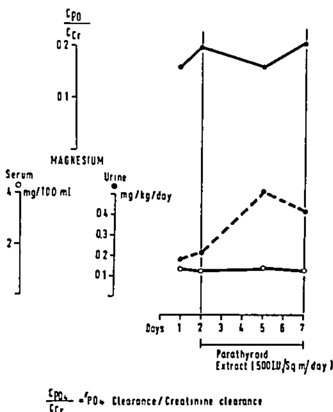


Fig 1 Ellsworth Howard test in patient with primary hypomagnesemia

Table 2 *Calcium metabolism of the patient with primary hypomagnesemia studied by* ^{47}*Ca i-v injection*

	Patient	Normal children
Exchangeable pool (mg/kg)	75 5	139±46
Accretion (mg/kg/day)	53 7	46 7±12 2
Urinary elimination (mg/kg/day)	0 3	4±2
Intestinal elimination (mg/kg/day)	3 2	3 18±1 06

demonstrated a reduced exchangeable pool low urinary excretion normal values of accretion and no intestinal hypersecretion of Ca (Table 2)

On normal diet with plasma phosphorus between 5 2 and 7 1 mg/100 ml urinary phosphorus fluctuated between 10 7 and 36 mg/kg/day and phosphorus renal clearance was not significatively modified (tubular reabsorption 81–91%)

On Mg therapy the normalization of plasma phosphorus corresponded to a lowering of the urinary phosphorus (5 1–8 3 mg/kg/day)

The injection of parathyroid extract (Ellsworth Howard test) performed 25 days after Mg therapy was discontinued (plasma calcium 10 6 mg/100 ml plasma phosphorus 7 mg/100 ml plasma magnesium 0 32 mg/100 ml) did not provoke any modification of the phosphorus renal clearance/creatinine renal clearance ratio (Fig 1) Blood Mg level was unmodified while urinary magnesium was increased by parathyroid extract (Fig 1)

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Patient on normal diet	2 677 50	1 281 00	25 25	+1 371 25	+52 15	
	2 677 50	1 222 50	20 98	+1 434 02	+54 34	0 32
	2 677 50	1 396 50	22 75	+1 258 25	+47 84	0 00
Mean value	2 677 50	1 300 00	22 99	+1 354 5	+51 44	0 16
Patient on Mg supplementation						0 00
Control subjects on normal diet	2 655 00	985 85	85 15	+1 584 00	+62 87	52 4±18
Control subject on experimental[a] Mg deficiency						46 29

[a] 47 days of low Mg diet

Howard test was also performed In the schema (Fig 2) columns indicate the 24-hours urinary excretion of P and Ca the dotted line indicates the plasma calcium values before and after 3 hours of calcium perfusion and the continuous line indicates the 24-hours phosphorus renal clearance before and after calcium perfusion

Without Mg therapy (plasma calcium 6 5 mg/100 ml plasma phosphorus 7 mg/100 ml plasma magnesium 0 32 mg/100 ml) no significative modifications of renal output of calcium and phosphorus were observed sma calcium increased and Ca retention s practically 100 On Mg therapy the re ction of phosphorus renal clearance was ry evident and the high Ca retention per ted (98%)

X ray findings did not demonstrate significant evidence of bone mineralisation (femur and tibia metaphysis are a little thickened) Serum alkaline phosphatase was always within normal range (35–89 i u)

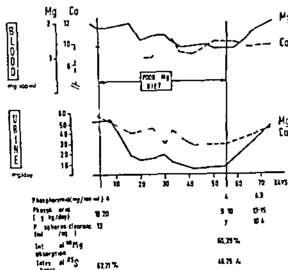


Fig 3 Modification of biochemical data in a control child on a low Mg diet

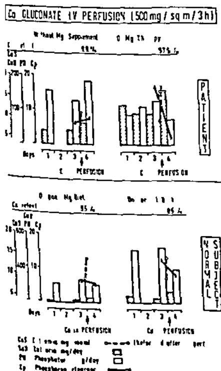


Fig 2 Howard test in patient with hypomagnesemia, on a normal Mg intake then and on Mg supplements and in a control subject, on low Mg intake and then on normal diet.

Ca P Sr and Mg metabolism in control children fed with either low Mg diet or rich Mg diet

In a boy fed with a prolonged low Mg diet with normal Ca supply urinary magnesium fell quickly Plasma magnesium also fell but slower and to a lesser degree Intestinal absorption of ²⁸Mg was raised. It was much higher than in the patient with primitive hypomagnesemia (Fig 3 Table 1 of the previous paper)

Plasma calcium was practically unchanged but urinary calcium fell ⁸⁵Sr intestinal absorption also fell but it did not attain the low values found in the patient with primary chronic hypomagnesemia (Table 1) Urine phosphorus excretion and phosphorus renal clearance fell without any significant modification of plasma phosphorus (Fig 3)

In this boy Howard test was also performed on normal diet and on low Mg diet On a low Mg diet the P renal clearance diminished slightly and the Ca body retention was high When a normal diet was given again for 15

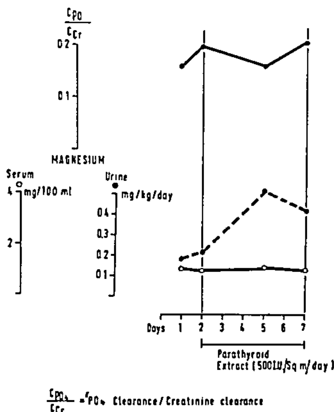


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high) and the Mg glycerophosphate supply favours the absorption of both ^{47}Ca and ^{8} Sr but chiefly of the latter (Figs 3 and 4)

Of course there are many data which emphasize that more than one transport mechanism exists for these cations It seems that Mg and Sr are linked to each other more than Mg and Ca at intestinal transport level

The influence of Mg on Ca P homeostasis

The interpretation of this influence is particularly difficult Both in primary chronic hypomagnesemia and experimental human (8 35 personal observation Fig 3), puppie baby pig and calf (15 16 20) Mg deficiency plasma calcium and/or urinary calcium levels ar low In our case of primary Mg malabsorption and in Salet s one (31) this lowering is associated to a reduced exchangeable pool of Ca In both cases no hypersecretion of Ca into the intestine is demonstrated and the malabsorption of exogenous Ca is not so much reduced as to explain such a severe alteration of Ca homeostasis

In our own and in three other cases (8 31, 36) there is also high plasma phosphorus but according to other authors (9 16 19 25 27 31) three arguments are against primitive hypoparathyroidism

1 although a Mg-deficiency is demonstrated by Gill et al (10) in hypoparathyroidism magnesemia is always exceedingly low

2 parathyroid extract does not correct hypomagnesemia

3 Mg therapy completely cures hypoparathyroid like symptoms Moreover phosphorus renal clearance is not clearly reduced

Secondary hypoparathyroidism is also unlikely since in calves (16) and sheeps (3) an inhibition instead of an activation of parathyroid function by Mg has been stressed

On the contrary our patient shows a hyposensitivity to exogenous parathyroid extract and vitamin D (first phase of the course of the disease and Ellsworth Howard test) and to the fluctuation of endogenous parathormone induced by the Ca perfusion (Howard test) (Figs 1 and 2 and Fig 1 of the previous paper) Such a hyposensitivity was also demonstrated in the case of Paunier et al (25) in human hypoparathyroidism with econdary Mg deficiency (18) and in Mg-deficient hypocalcemic calves (16) Moreover the role of Mg in regulating the sensitivity to parathormone may be confirmed by the results obtained with the Ellsworth Howard test in the boy fed with a low Mg diet (Fig 2)

On the contrary in the case of primary hypomagnesemia of Strømme et al (36) the increase of plasma calcium after parathyroid extract treatment was greater than in control subjects

In our case increased urinary magnesium without any significant modification of plasma magnesium on parathyroid extract treatment (Fig 1) may be interpreted as a consequence of a mobilisation of residual Mg from bones and contrasts with the Mg tubular reabsorption stimulating function of parathormone (19)

Normal Ca accretion in our case and in Salet s one contrasts with the reduced exchangeable Ca pool (Table 1) but is compatible with good bone mineralisation demonstrated by both Howard test and X ray findings

In hypocalcemic Mg-deficient calves Larvor et al (16) demonstrated a low mineral dynamic Considering that pyrophosphatase which is certainly involved in the regulation of bone mineral dynamic is Mg-dependent and the fact that Mg increases Ca removal from bone in parathyroidectomized animals and cures experimental hypoparathyroidism (4 6) an impairment of local factors regulating bone mineral turnover cannot even be excluded

Variable interrelations may exist between the degree of intestinal Mg and Ca absorption parathormone production parathormone and vitamin D sensitivity and the Mg-dependent bone factors regulating mineral dynamic in the different cases of chronic idiopathic hypomagnesemia with secondary hypocalcemia hyperphosphoremia

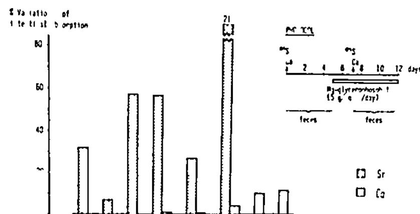


Fig 4 The influence of oral Mg glycerophosphate on ⁸⁵Sr and ⁴⁷Ca intestinal absorption

days (plasma magnesium 1 8 mg/100 ml) the reduction of the P clearance was more evident and the retention of Ca on Ca intravenous perfusion diminished a little (Fig 2)

In a few other control children the supplementation of the diet with high dose of Mg glycerophosphate provoked a more or less evident increase of the intestinal absorption of ⁸⁵Sr ⁴⁷Ca intestinal absorption was also increased but this effect of the Mg supplement was less marked and occurred only in 2 of the 5 studied children (Fig 4)

DISCUSSION

The investigation performed in the patient raises the problem of the relationship between Mg, Ca and Sr in intestine and the influence of the alteration of Mg metabolism on Ca P homeostasis

The research carried out on control subjects allows us a better interpretation of this problem

Therefore all the results we obtained will be discussed conjointly

The relationship between Mg Ca and Sr absorption

In the patient with Mg malabsorption Ca balance is more positive than Mg balance as in other similar cases (9 25 31) and although balance data must be interpreted with caution, intestinal Ca absorption cannot be considered as certainly reduced (Table 1) On the contrary a very low ⁸⁵Sr absorption exists (Table 1) Of course in this case the relationship between Mg Ca and Sr at intestinal transport level seems to be rather complex

Many authors (1, 2 14 21, 24 33 34) stress the competition between Ca Mg and Sr for a common transport pathway Ogata et al (24) postulate that factors such as parathormone, which activate Ca transport primitively act on Mg transport Hendrix et al. (14) emphasize on the contrary that besides a common transport pathway there are specific mechanisms for the transport of these cations Laster & Ingelfinger (17) Ross (29) and Strømme et al (36) postulate that a saturating active process or a facilitated diffusion are involved in the transport of Mg Rasmussen & Ogata (26) stress that Mg and Ca transport depends on an unique mitochondrial source of energy but that two carriers are involved in this transport Heaton et al (13) emphasize a common pathway for Ca and Mg absorption but they state that vitamin D acts more on the former than on the latter Clark (5) demonstrated that Mg supply increases or reduces intestinal Ca absorption according to the Ca content of the diet Moreover although it is generally admitted that Sr transport parallels the Ca one a few observations show that differences exist in discrimination and transport speed of these cations in intestine and kidney (5 7 11 12 14 32)

The complex relationship between Mg Ca and Sr at intestinal level are also demonstrated by the research we performed in normal children A prolonged low Mg diet reduces the ⁸⁵Sr intestinal absorption (²⁸Mg absorption is

high) and the Mg glycerophosphate supply favours the absorption of both ^{47}Ca and ^{8} Sr but chiefly of the latter (Figs 3 and 4)

Of course there are many data which emphasize that more than one transport mechanism exists for thes cations It seems that Mg and Sr are linked to each other more than Mg and Ca at intestinal transport level

The influence of Mg on Ca P homeostasis

The interpretation of this influence is particularly difficult Both in primary chronic hypomagnesemia and experimental human (8 35 personal observation Fig 3) puppie baby pig and calf (15 16 20) Mg deficiency plasma calcium and/or urinary calcium levels are low In our case of primary Mg malabsorption and in Salet s one (31) this lowering is associated to a reduced exchangeable pool of Ca In both cases no hypersecretion of Ca into the intestine is demonstrated and the malabsorption of exogenous Ca is not so much reduced as to explain such a severe alteration of Ca homeostasis

In our own and in three other cases (8 31 36) there is also high plasma phosphorus but according to other authors (9 16 19 25 27 31) three arguments are against primitive hypoparathyroidism

1 although a Mg-deficiency is demonstrated by Gill et al. (10) in hypoparathyroidism magnesemia is always exceedingly low

2 parathyroid extract does not correct hypomagnesemia

3 Mg therapy completely cures hypoparathyroid like symptoms Moreover phosphorus renal clearance is not clearly reduced

Secondary hypoparathyroidism is also unlikely since in calves (16) and sheeps (3) an inhibition instead of an activation of parathyroid function by Mg has been stressed

On the contrary our patient shows a hyposensitivity to exogenous parathyroid extract and vitamin D (first phase of the course of the disease and Ellsworth Howard test) and to the fluctuation of endogenous parathormone induced by the Ca perfusion (Howard test) (Figs 1 and 2 and Fig 1 of the previous paper) Such a hyposensitivity was also demonstrated in the case of Paunier et al (25) in human hypoparathyroidism with secondary Mg-deficiency (18) and in Mg deficient hypocalcemic calves (16) Moreover the role of Mg in regulating the sensitivity to parathormone may be confirmed by the results obtained with the Ellsworth Howard test in the boy fed with a low Mg diet (Fig 2)

On the contrary in the case of primary hypomagnesemia of Strømme et al (36) the increase of plasma calcium after parathyroid extract treatment was greater than in control subjects

In our case increased urinary magnesium without any significant modification of plasma magnesium on parathyroid extract treatment (Fig 1) may be interpreted as a consequence of a mobilisation of residual Mg from bones and contrasts with the Mg tubular reabsorption stimulating function of parathormone (19)

Normal Ca accretion in our case and in Salet s one contrasts with the reduced exchangeable Ca pool (Table 1) but is compatible with good bone mineralisation demonstrated by both Howard test and X ray findings

In hypocalcemic Mg-deficient calves Larvor et al (16) demonstrated a low mineral dynamic Considering that pyrophosphatase which is certainly involved in the regulation of bone mineral dynamic is Mg-dependent and the fact that Mg increases Ca removal from bone in parathyroidectomized animals and cures experimental hypoparathyroidism (4 6) an impairment of local factors regulating bone mineral turnover cannot even be excluded

Variable interrelations may exist between the degree of intestinal Mg and Ca absorption parathormone production parathormone and vitamin D sensitivity and the Mg-dependent bone factors regulating mineral dynamic in the different cases of "chronic idiopathic hypomagnesemia with secondary hypocalcemia hyperphosphoremia

CONCLUSIONS

Magnesium in the diet plays an important role in regulating Ca and Sr absorption

Probably a part only of the mechanism regulating the absorption of these cations is impaired in primary hypomagnesemia and one cannot exclude that it mainly regulates Mg and Sr passive transport

Mg deficiency secondary to Mg malabsorption, provokes an alteration of Ca P homeostasis Parathormone and vitamin D resistance is clear enough but the whole pathogenesis of the alteration of Ca P homeostasis is unknown and further investigation is necessary to understand it better

SUMMARY

A study has been carried out on the relationship between M Ca and Sr in a case of primary Mg malabsorption and in control children on high and low Mg diets

Probably more than one transfer system exists for these cations in intestine and in Mg-malabsorption the simple diffusion of both Mg and Sr is impaired

The influence of Mg on Ca P homeostasis is not clearly understood but a resistance to parathormone and vitamin D in Mg deficiency is emphasized

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PIMARICIN (NATAMYCIN) IN THE TREATMENT OF SUPERFICIAL FUNGAL INFECTIONS IN CHILDREN

Z LASKOWNICKA K PASYK A PORĘBSKA and K ZEMBUROWA

From the Paediatric Institute and the Department of Medical Microbiology Medical Academy Kraków Poland

Among the relatively few antifungal antibiotics which have found practical application in medical treatment, pimaricin (natamycin) is prominent because of its wide spectrum of activity against not only yeast-like fungi, but also moulds and dermatophytes and Trichomonas vaginalis (1, 7 10, 18, 21, 22) Discovered in 1955 by Struyk and coworkers (27), it is produced by Streptomyces natalensis Its tetraen structure is similar to but not identical with nystatin (20) In view of its wide spectrum of activity it aroused the interest of clinicians and was applied first of all in dermatology and gynecology (2–6, 11–14 16 19, 23, 25, 26 28,29)

Since the absorption of pimaricin in the intestinal tract is poor, it is used mainly locally Oral tablets may be used complementary to local treatment Pimaricin is available in various preparations suitable for the treatment of different fungal infections

PROCEDURE

This report concerns clinical studies of four preparations of pimaricin[1] pimafucin suspension 1 pimafucin vaginal tablets pimafucin ointment and pimafucort ointment The patients considered in the study were treated in the Paediatric Institute Medical Academy in Kraków They were in part hospitalized and in part treated as out patients A total of 69 children and young people up to age eighteen were treated Among them there were 59 children up to age fourteen and ten from age fourteen to eighteen

Twenty four patients with vaginitis were treated with pimafucin vaginal tablets Among them there were 15 girls aged eight to fourteen and 9 older girls aged fifteen to eighteen years Sixteen patients were previously treated without success with various antifungal preparations and antiseptics including nystatin marcostatin borax with glycerin gentian violet and sterovag One patient was previously treated with metranidazole and one with sigmamycin In this group of patients symptoms persisted for a long time ranging from 3 months to 7 years Nystatin was previously applied without success in 9 of these cases In 8 patients previously untreated symptoms appeared from 3 weeks to 14 months earlier The applied treatment with pimafucin vaginal tablets was local and included one or two courses each lasting 20 days Every night before retiring one tablet cut along into two pieces was introduced into vagina by means of narrow forceps Disappearance of clinical symptoms and negative results of three mycological examinations performed after 1 4 and 8 weeks after discontinuation of the therapy were considered as complete cure Cases of vulvovaginitis in which complete cure was not achieved were submitted to the second course of treatment

Fourteen children aged from 2 days to 12 years suffering from oral thrush or stomatitis with secondary yeast like fungi infections were treated with pimafucin suspension 1 Four to five drops of the drug were given under the tongue or to the lesion itself in small children and seven to ten drops in older children

Thirty one children with different skin infections as angulus infectiosus intertrigo otitis externa candidiasis unguium were treated with pimafucin or pimafucort ointments The treatment consisted of direct application of ointment lightly rubbed in twice or several times daily sometimes under dressing during 5 to 105 days

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Table I *Results of treatment with different pimaricin preparations in children*

Diagnosis	Total number examined	Pimaricin preparation applied	Results of treatment: Cure	Improvement	No success
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Candidiasis mucosae oris	8	Pimafucin suspension 1	4	1	3[b]
Stomatitis aphtosa (with secondary yeast like fungi infection)	3	Pimafucin suspension 1	3	—	—
...atitis ulcerosa ...th secondary yeast ... fungi infection)	3	Pimafucin suspension 1	1	2	—
...ulus infectiosus oris	3	Pimafucin ointment	2	1	—
...initis blastomycetica	1	Pimafucin ointment	1	—	—
...rtrigo regionis ...nitalis blastomycetica	3	Pimafucin ointment	2	1	—
...ndidiasis unguium	3	Pimafucin ointment	2	1	—
...gulus infectiosus oris	4	Pimafucort ointment	3	1	—
...tis externa chronica	3	Pimafucort ointment	3	—	—
...ertrigo regionis ...italis blastomycetica	11	Pimafucort ointment	11	—	—
...ndidiasis unguium	3	Pimafucort ointment	1	2	—
...tal	69		53	12	4
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RESULTS

The results of treatment of different mycotic infections by means of four studied pimaricin preparations are presented in the table In the cases of vulvovaginitis Candida albicans was found in 13 patients other species of Candida (C krusei C parakrusei, C tropicalis C pseudotropicalis) in 6 Torulopsis sp in 2 Double infection with Candida albicans and Geotrichum sp was shown in one patient and in two there was double infection with moulds (Penicillium sp and Scopulariopsis sp) In 45 other cases of different fungal infections Candida albicans was found in 32 Candida krusei in one Rhodotorula sp in 2 and Aspergillus fumigatus in 2 Mixed infections with different species of Candida and Geotrichum sp (C albicans + C Tropicalis C albicans + C parakrusei C pseudotropicalis + Geotrichum sp C. albicans + C pseudotropicalis + Geotrichum

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tions Complete cure was achieved after only one course of treatment with pimafucin vaginal tablets Excellent results were obtained in the treatment of otitis externa chronica which was also observed by Alteras (1) In these cases the lesions w re healed within 10–14 days by the application of pimafucort ointment

SUMMARY

Sixty nine children suffering from superficial fun al infections were treated with four pimari cin preparations pimafucin vaginal tablets pimafucin 1° suspension pimafucin and pimafucort ointments Complete cure was achieved in fifty three children (76 8°) improvement in twelve (17 3%) In four (5 7%) children with serious basic diseases (leukaemia diabetes) the treatment was unsuccessful No signs of intolerance were observed

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sp) were detected in 5 patients In 3 cases mycological examinations were not performed

Among the cases of vulvovaginitis in 16 patients full clinical cure with three negative cultures was achieved after one course of treatment Eight other patients were submitted to the second course of treatment and cure was achieved this time in 4 cases, with improvement in 3 other cases In one case only the treatment failed even after the third course It should be pointed out that it was a case of poorly controlled juvenile diabetes mellitus Two other girls who improved after the second course of treatment suffered from diabetes mellitus too Summarizing all the cases of vulvovaginitis treated with pimafucin vaginal tablets, cure after one or two courses was achieved in 20 girls and improvement in 3

Pimafucin suspension 1% applied for the treatment of lesions localized in mouth resulted in complete cure of 8 patients, there was improvement in 3 cases and in 3 others the treatment was unsuccessful In the latter group, 2 children were suffering from leukaemia and were in poor general condition

After the therapy with pimafucin or pimafucort ointments applied in 31 children cure was achieved in 25 cases and improvement in 6

Considering all the results of treatment with different pimaricin preparations totalling 69 children, cure was noted in 53 (76 8%) and improvement in twelve (17 3%) In four (5 7%) children mainly with serious diseases treatment was unsuccessful In no case was any sign of intolerance observed

DISCUSSION

Recently there have appeared many reports on advantageous curative effects of pimaricin in fungal infections of skin and mucous membranes (9, 23) The effectiveness of the new antibiotic was emphasised especially in infections caused by yeast like fungi of Candida species The latter are wide spread in nature and have an important position in human pathology, particularly Candida albicans which is closely linked with living human or animal organisms (8) It may be present in normal mucous membranes of the mouth and vagina, hence the first contact with this microorganism in newborn children occurs in the genital tract of the mother An excessive multiplication of microorganisms and in consequence development of illness takes place in the conditions of resistance deficiency such as blood diseases, metabolic disturbances, or during prolonged therapy with antibacterial broad spectrum antibiotics or corticosteroids Candida infections often occur in children and are dangerous especially for infants whose mechanisms of natural resistance are not fully developed (29) Hence in this report, dealing with children Candida infections dominated and were observed in the form of stomatitis, oral thrush angulus infectiosus and intertrigo Vulvovaginitis caused by yeast like fungi is not rare in girls during puberty and may present a serious therapeutical problem

Not many papers published so far have dealt with the application of pimaricin in children. In this respect the report of Van Den Driessche & Bottu (29) is noteworthy The authors treated successfully with pimaricin skin and intestinal tract candidiasis in small children Much attention was paid to the treatment of vulvovaginitis of mycotic or protozoal etiology (4 6, 12 13 17 19, 22–24), and further attention was given to the problem of treatment vulvovaginitis in pregnant women where the elimination of Candida infection is of particular importance in view of possible infection of the newborn child (35) The results of our attemptive treatment of vulvovaginitis in girls correspond to those obtained by other authors in the treatment of adult women and assured satisfactory percentage of healed cases A noteworthy case of a 17 year old girl suffering from persistent vulvovaginitis since the age of ten was observed She had been treated unsuccessfully with nystatin borax with glycerin, gentian violet and sterovag Before starting that treatment with pimaricin Candida pseudotropicalis was cultured from her vaginal secre-

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DIETARY COMPOSITSION AND DENTAL DISEASE IN ADOLESCENT DIABETICS

A pilot study

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Th relationship b tween diabetes mellitus and d ntal health has long b en a matter of disputa tion The current opinion seems to be that diabetes has a modifying effect on the in cidence of dental caries (8) and an accelerating effect on locally initiated periodontal disease (4) There is suggestive evidence that dietary habits play an important role in the pathogene sis of dental caries (2 13) and in the develop ment of supragingival calculus (4 5 9)

Two cross sectional surveys conducted with a five year interval of dietary intakes among adolescent diabetics (10 12) revealed that they consumed a higher protein and lower carbo hydrate percentage of total calories than non diab tics controls Those factors favouring den tal health among nondiabetics (2 15) might be counteracted by the prevalence of continu ously elevated blood glucose and probably salivary glucose concentration in most juvenile diabetics (4 11) It thus seemed of interest to compare directly the dietary composition with th result of a dental examination in groups of diabetics and nondiabetics

MATERIAL AND METHODS

The experimental groups were part of a larger popula tion und r cont nuous follow up in estigation (11 1) The material consisted of 41 subjects, 24 d abetics and 17 nond abetics who attended the follow up [illegible] termined period of 3 weeks. They were below 25 years and were living in the city of Stockholm None of the subjects had knowl edge in advance about the dental examination to be performed

The dietary intakes were assessed by the 24-hour recall method The interviews were planned con ducted and the dietary composition calculated as previously described (10) Each subject was inter viewed twice with 10–20 days intervals the second recall at the day of dental examination During the week before the dental examination all subjects also kept a dietary record

Dental caries was quantitated in each case by the 100-surface index (14) and evaluated according to an established method using simultaneous technique (6) A similar technique was used in the registration of dental calculus of supragingival type (4) Oral hygiene was recorded at the second interview as the number of tooth brushings per day

The degree of diabetic control was evaluated in each patient as previously described (11) using mean degree of glycosuria over 24 hours and fasting blood glucose values at outpatient visits over a period of 5 years

RESULTS

The diab tics had a mean age of 17 4 years (range 13–19 yr) and the nondiabetics 17 3 years (range 15–23 yr) The heights and weights for each subject fell within ±1 S D for normals as did the weight/height ratio The mean age at onset of diabetes was 7 5 years (range 2–13 yr) and the mean duration 9 8 years (range 5–18 yr) The controls were chosen according to the "social twin" prin ciple

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nificant correlations were observed The dura tion of diabetes was not obviously related to dental caries

COMMENTS

The general pattern of lower intake of refined carbohydrates taken less frequently among the diabetics than among the nondiabetics could well play a part in explaining the tendency to a lower frequency of dental caries among dia ·s (1 2 3) A minor significance would be given to elevated blood glucose levels he formation of calculus is due to calcifica of dental plaque material Sucrose is an ellent substrate for some acid producing roorganisms also essential for the plaque lding process (1 7) Since the diabetics con ed relatively little refined carbohydrates nly sucrose they would have a lower fre ncy of supragingival calculus than the non betics However the higher protein content the diabetic diet might increase the buffer capacity in mixed saliva i e the capacity to nteract pH-changes A high buffering capa v has been suggested to favour calculus mation among nondiabetics (2 5 9 15) d might thu explain the somewhat higher quency of calculus among the diabetics in is material

A true picture of the degree of diabetic con ol over a long period of many years will prob- bly never be obtained (11) Recognising that e available parameters are at best only es mates of diabetic control it is not surprising hat in the present small material no correla ion to dental health was found

The 24 hour recall method had good repro- ucibility (Table 1) both in the diabetic and he nondiabetic group On the other hand there was a considerable decrease in the subjects own reported caloric intake in comparison with the results of interviews consistent with reports in normal individuals (16) The most obvious drop was found in the consumption of refined carbohydrates among the diabetics suggesting that self recording methods should not be used for nutritional surveys in subjects who are pre- scribed specific diets

SUMMARY

In a group of adolescent diabetics there was a probable lower frequency of caries but a higher frequency of supragingival calculus than in the control group The diabetics consumed relatively more protein and less refined carbo- hydrates taken less frequently than nondia betics The 24 hour recalls showed good re producibility but an unsatisfactory correspond- ance to a 7 day record among diabetics

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Table 1 *Comparison between mean dietary intakes at two "24 hour recalls" (I and II) and one 7-day record (III)*

No of cases	Calories I	Calories II	Calories III	Protein (g and °) I	Protein II	Protein III	Fat (g and) I	Fat II	Fat III	Carbohydrate (g and) I	Carbohydrate II	Carbohydrate III	Refined carbohydrate (% of total) I	Refined carbohydrate II	Refined carbohydrate III
Diabetics															
15 boys	2 782	2 705	2 303	94 8	98 1	93 5	132 1	128 2	106 9	285 0	269 7	224 1			
				14	15	17	43	43	43	44	43	40	7	6	2
6 girls	1 666	1 747	1 583	57 5	66 7	62 3	81 7	78 0	75 4	164 4	184 2	148 4			
				14	15	17	45	41	44	42	44	41	4	5	3
Non diabetics															
11 boys	2 858	2 810	2 484	86 7	93 5	84 5	129 6	123 9	107 8	318 5	310 0	298 6			
				12	14	14	41	41	39	46	46	49	11	10	11
4 girls	2 195	2 166	1 848	74 8	82 5	63 7	113 3	104 7	88 3	204 6	209 1	194 2			
				13	15	14	44	43	44	43	43	42	8	7	10

A careful inspection of the individual caloric intakes among diabetics and nondiabetics, divided according to age and sex, suggested that the present material as regards dietary composition would be representative for the whole group followed up

The mean consumption of calories and the percentage distribution of protein fat and carbohydrate are given in Table 1 for those 36 subjects who completed the three methods of assessing dietary composition Refined carbohydrates (Table 1) were consumed on the average 0 8 times a day among diabetics and 1 8 times a day among nondiabetics

The two 24 hour recalls showed very similar mean dietary consumptions within each group The 7 day record gave lower caloric intake both among diabetics and non diabetics but an unchanged distribution on protein fat and carbohydrate The percentage of refined carbohydrates was considerably lower in the diabetics own records than that obtained by the interviews No such difference was seen among nondiabetics

The diabetic group had a somewhat lower caloric intake but a higher intake of protein in per cent of total calories than the comparable nondiabetic group On the other hand the proportion of refined carbohydrates was lower among diabetics than among nondiabetics irrespective of the method of assessment.

The results of the dental examination are given in Table 2 The diabetics had a lower frequency of caries but a higher frequency of supraingival calculus than the nondiabetics Oral hygiene was similar in both groups

The mean glycosuria was 28 g/25 hours (range 13–41) among diabetic boys and 35 g/24 hours (13–53) among diabetic girls The mean fasting blood glucose was 280 mg/100 ml (100–430) among the boys and 330 mg/100 ml (230–490) among the girls The individual data were plotted against the frequency of caries and calculus, but no sig

Table 2 *Dental status among diabetics and controls*
Mean values ± S D For units see text

Material	No of cases	Oral hygiene	Dental caries	Dental supragingival calculus
Diabetics	24	2 00±0 49	24 35±12 47	5 41±5 57
Controls	17	2 23±1 16	34 37±16 73	2 37±3 69
Significance		n s	$p<0\ 05$	$p<0\ 05$

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FIBRINOGEN TURNOVER IN THE PREMATURE INFANT WITH AND WITHOUT IDIOPATHIC RESPIRATORY DISTRESS SYNDROME

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From the Children s Hospital and the Blood Transfusion Unit University of Freiburg i Br Western Germany

The physiology and pathology of blood coagulation in the newborn and premature infant have gained in interest since a number of authors described intravascular fibrin clots in histological sections of various organs of infants dying in the neonatal period These clots were considered to be the result of intravital intravascular coagulation (3 5 6)

The hypothesis originally set up by Stark et al (13) of a correlation between intravascular coagulation (i v C) and idiopathic respiratory distress syndrome (IRDS) has been the subject of research in this hospital In a series of programmes so far carried out no significant diminution of those factors usually considered to be consumed during i v C (I II V VII and platelets) could be demonstrated in uncomplicated IRDS (7 10) In those infants who died of IRDS however these factors were diminished A feasible explanation for this discrepancy betwe n almost normal coagulation in infants with uncomplicated IRDS on the on hand and depression of coagulation po ntial with intravascular fibrin clots in infants dying of IRDS on the other would be that premature infants are able to compensate rapidly for loss of coagulation factors Factor I fibrinogen app ars particularly suitable for the investigation of this hypothesis as it is available as a pure substance tagged with radio-active ^{125}I for half lif determination studies

MATERIALS AND METHODS

Patients

Ten premature infants aged between 1 and 9 hours were studied Every infant received a small quantity of potassium iodide paste applied to the skin before the injection of ^{125}I fibrinogen and on 3 subsequent days in order to block the uptake of ^{125}I by the thyroid gland

4 of the infants were healthy at the time they were chosen for the test and remained so (C 1380 g B 1880 g D 1900 g O 2430 g) One of these (O) was not considered for calculation of fibrinogen half life as it was discharged before the end of the test

Six infants (K 1450 g Ma 2340 g Mu 1800 g S 1670 g N I and N II 1300 g) had the characteristic clinical and X ray symptoms of IRDS and were treated in the usual manner with high ambient oxygen concentrations and infusion of glucose solutions containing sodium bicarbonate for correction of acidosis according to serial micro-Astrup measurements Two of these infants twins (N I and N II) died before the fourth day of life For comparison the half life of ^{125}I tagged fibrinogen was calculated in an adult weighing 92 kg

METHODS

^{125}I Fibrinogen A commercial product[1] was used (specific activity = 0 15 mCi mg) In contrast to ^{131}I which has been most used hitherto ^{125}I is a soft γ ray emitter free of β radiation The radiation dose for the tissues is therefore lower even taking into account the longer physical half life The premature infants received 5–15 μCi, the adult 85 μCi of ^{125}I fibrinogen applied intravenously

Plasma fibrinogen determination Blood sampling

[1] Swiss Institute for Reactor Research CH Würenlingen

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The *hypothesis* originally set up by Stark et al. (13) of a correlation between intravascular coagulation (i.v.C.) and idiopathic respiratory distress syndrome (IRDS) has been the subject of research in this hospital. In a series of programmes so far carried out no significant diminution of those factors usually considered to be *consumed* during i.v.C. (I, II, V, VII and platelets) could be demonstrated in uncomplicated IRDS (7, 10). In those infants who died of IRDS, however, these factors were diminished. A feasible explanation for this discrepancy between almost normal coagulation in infants with uncomplicated IRDS on the one hand and depression of coagulation potential with intravascular fibrin clots in infants dying of IRDS on the other would be that premature infants are able to compensate rapidly for loss of coagulation factors. Factor I (fibrinogen) appears particularly suitable for the investigation of this hypothesis as it is available as a pure substance tagged with radio-active ^{125}I for half life determination studies.

MATERIALS AND METHODS

Patients

Ten premature infants aged between 1 and 9 hours were studied. Every infant received a small quantity of potassium iodide paste applied to the skin before the injection of ^{125}I fibrinogen and on 3 subsequent days in order to block the uptake of ^{125}I by the thyroid gland.

4 of the infants were healthy at the time they were chosen for the test and remained so (C. 1 380 g, B. 1 880 g, D. 1 900 g, O. 2 430 g). One of these (O) was not considered for calculation of fibrinogen half life as it was discharged before the end of the test.

Six infants (K. 1 450 g, Ma. 2 340 g, Mu. 1 800 g, S. 1 670 g, N I and N II 1 300 g) had the characteristic clinical and X-ray symptoms of IRDS and were treated in the usual manner with high ambient oxygen concentrations and infusion of glucose solutions containing sodium bicarbonate for correction of acidosis according to serial micro-Astrup measurements. Two of these infants, twins (N I and N II), died before the fourth day of life. For comparison the half life of ^{125}I tagged fibrinogen was *calculated* in an adult weighing 9[illegible] kg.

METHODS

^{125}I Fibrinogen. A commercial product[1] was used (specific activity ≈ 0.15 mCi/mg). In contrast to ^{131}I which has been most used hitherto, ^{125}I is a soft γ-ray emitter free of β radiation. The radiation dose for the tissues is therefore lower even taking into account the longer physical half life. The premature infants received 5–15 μCi, the adult 85 μCi of ^{125}I fibrinogen applied intravenously.

Plasma fibrinogen determination. Blood sampling

[1] Swiss Institute for Reactor Research, CH Würenlingen

Table 1. *Biological half life and calculated catabolic rate for* ^{125}*I fibrinogen in the adult and premature infant. Changes in total plasma fibrinogen concentration during the first days of life in premature infants*

Mean values and standard deviations were not calculated for the fibrinogen concentrations in this small series. For dynamics of plasma fibrinogen in the premature infant during the first day of life see Karitzky et al. (10)

	Adult	Normal premature infants ($n=3$)			Premature infants with IRDS surviving ($n=4$)			
Half life (days)	4.5	2.8			2.1			
Catabolic rate (turnover/day) in percent	15.0	25.2			33.0			
		C	B	D	K	Ma	Mu	S
Total plasma fibrinogen (immunologically determined) in mg/100 ml before injection of ^{125}I fibrinogen	265	182	325	150	191	200	190	370
After 24 hours	273	220	333	158	240	208	226	320
After 3 days	273	253	360	188	273	226	194	416
After 7 days	273	234	355	168	260	226	194	468
After 12 days	273	235	334	200	290	234	208	460

was done at 15 and 30 min, and at 1, 6, 12 and 24 hrs after injection, later at daily intervals. 1.5 ml aliquots of blood were heparinised with an unweighed lyophilised crystal of ammonium heparinate (ROCHE). Fibrinogen determinations were carried out by radial Immunodiffusion (9, 11).

^{125}*I fibrinogen determination.* 0.25 ml of plasma were incubated for 2 hrs at 37 C, then for 24 hrs at 4 C with 0.2 ml monospecific anti human fibrinogen serum from the rabbit (8). The resulting immunoprecipitate was centrifuged at 15 000 g for 2 min and washed three times in buffered physiological saline. The radioactivity of all the precipitates was measured at the end of each experiment with a Packard Auto-Gamma Spectrometer (Model 3575). The activity 30 min after application of the ^{125}I fibrinogen was taken as 100%. The other measurements were expressed as ratios of this value after correction for zero emission.

External body measurements. For measurements over various organs a shielded Geiger Muller tube was used. In this way background interference was reduced four fold as compared with a scintillation counter. The detector tube was connected to a digital counter, the assay time was preset according to the expected impulse rate. The body measurements were taken in the following four positions:

1. Over the heart. Third intercostal space centred 3 cm from the sternal border.
2. In the right axilla. Pointing exactly towards the opposite axilla.
3. Over the right thigh. Over the diaphysis of the right femur and at 90° to this.
4. Over the right temporal region. On the midline between external orbita and external auditory meatus pointing exactly towards the opposite temporal region.

Statistical calculations. Arithmetical means were calculated for the ^{125}I fibrinogen activities measured between the third and twelfth day after injection. After demonstration of highly significant differences between the fibrinogen activity curve characteristics in the adult, the healthy prematures and the prematures with IRDS, variance analyses were carried out on the IBM 7040 computer in the Dept. of Mathematics, University of Freiburg.[a]

RESULTS

Fate of ^{125}*I fibrinogen in adult*

85 μCi of ^{125}I fibrinogen were injected intravenously and plasma disappearance rate was measured in the manner described above. It can be seen (Fig. 1, diagram 1) that after an initial rapid fall in concentration, linear disappearance ensues at about 24 hours after injection (semi logarithmic scale). The half life is about 4.5 days or 108 hours, corresponding to a catabolic rate of 15% per day. This result compares well with recent results in the literature (1, 4) whereas McFarlane (12) gives 74 hours as being the normal value. Assuming that the steep fall in activity during the first day is

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[a] Our thanks are due to Dr Bloedhorn, Institute of Medical Statistics and Documentation, University of Freiburg.

due to the distribution into the extravascular compartment, the intravascular fraction would be 40 after equilibration This value is marked by the lower ordinate (the value is attained by extrapolation of the straight line during the second (steady) phase back to the ordinate)

¹²⁵I fibrinogen turnover in normal premature infants

The disappearance rate of ¹²⁵I fibrinogen in 3 normal premature infants can be seen in Fig 1 diagram 2 All results are plotted and the curve joints th mean values It can be seen that a steady fall in activity is not attained before the 2nd to 3rd day after injection From then on the function is a straight line when plotted on a semilogarithmic scale The half life is 2 8 days from the third day of life on correspond ing to a catabolic rate of 25 2 % p r day An assessment of fibrinogen metabolism during the first 3 days of life is not possible presumably

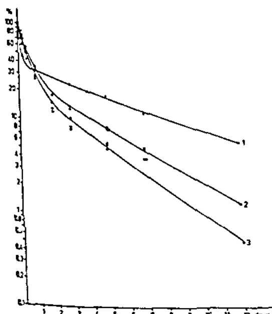


Fig 1 Rate of elimination of ¹²⁵I fibrinogen from the plasma in an adult (curve 1) in 3 normal premature infants (curve 2) and in 4 premature infants with IRDS surviving The curves 2 and 3 joint the mean values, abscissa Days after injection of ¹²⁵I fibrinogen ordinata. ¹²⁵I fibrinogen activity in the plasma (as percent of the 30 min-value)

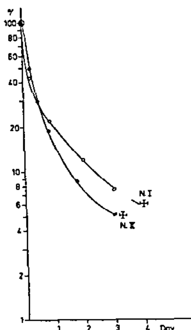


Fig 2 Rate of elimination of ¹²⁵I fibrinogen from the plasma of premature twins dying of IRDS (N I and N II) abscissa Days after injection of ¹²⁵I fibrinogen and days of life ordinata ¹²⁵I fibrinogen activity in the plasma (as percent of the 30-min value)

because a steady state is not reached Analysis of the curves permits the tentative assumption of two consecutive e functions but the scatter is too large for certainty

¹²⁵I fibrinogen turnover in infants with IRDS

Here again (Fig. 1 diagram 3) a steady state is not achieved before the third day of life The half life of fibrinogen is 2 1 days corresponding to a catabolic rate of 33% per day

Variance analysis

For the period after equilibration (stable phase) following the third day orthogonal comparisons were carried out There was a highly significant difference between the curves 1 (adult) and 2 (premature infants) (Fig 1) the test quotient in the F test being 20 49 for $p \leq 0.001$ The difference between curves 1 and 3 (premature infants with IRDS surviving) was also highly significant ($p \leq 0.001$) the test quotient being 32 14 However there was no difference between curves 2 and 3 (test quotient 2.15)

Table 1 *Biological half life and calculated catabolic rate for ¹ ⁵I fibrinogen in the adult and prem ture infant Changes in total plasma fibrinogen concentration during the first days of life in prematu infants*

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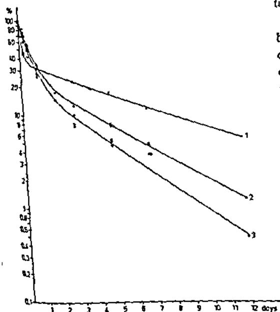


Fig 1 Rate of elimination of ¹²⁵I fibrinogen from the plasma in an adult (curve 1) in 3 normal premature infants (curve 2) and in 4 premature infants with IRDS surviving The curves 2 and 3 joint the mean values abscissa Days after injection of ¹²⁵I fibrinogen ordinata ¹²⁵I fibrinogen activity in the plasma (as percent of the 30 min value)

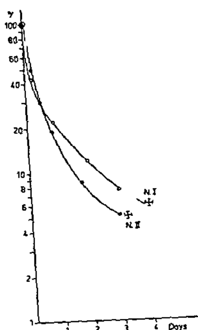


Fig 2 Rate of elimination of ¹²⁵I fibrinogen from the plasma of premature twins dying of IRDS (N I and N II) abscissa Days after injection of ¹²⁵I fibrinogen and days of life ordinata ¹²⁵I fibrinogen activity in the plasma (as percent of the 30-min value)

because a steady state is not reached Analysis of the curves permits the tentative assumption of two consecutive e functions but the scatter is too large for certainty

¹²⁵I fibrinogen turnover in infants with IRDS

Here again (Fig 1 diagram 3) a steady state is not achieved before the third day of life The half life of fibrinogen is 2 1 days corresponding to a catabolic rate of 33 per day

Variance analysis

For the period after equilibration (stable phase) following the third day orthogonal comparisons were carried out There was a highly significant difference between the curves 1 (adult) and 2 (premature infants) (Fig 1) the test quotient in the F test being 20 49 for $p \leq 0.001$ The difference between curves 1 and 3 (premature infants with IRDS surviving) was also highly significant ($p \leq 0.001$) the test quotient being 32 14 However there was no difference between curves 2 and 3 (test quotient 2 15)

Table 1 *Biological half life and calculated catabolic rate for ¹²⁵I fibrinogen in the adult and premature infant Changes in total plasma fibrinogen concentration during the first days of life in premature infants*

Mean values and standard deviations were not calculated for the fibrinogen concentrations in this small series For dynamics of plasma fibrinogen in the premature infant during the first day of life see Karitzky et al (10)

	Adult	Normal premature infants ($n=3$)			Premature infants with IRDS surviving ($n=4$)			
Half life (days)	4 5	2 8			2 1			
Catabolic rate (turnover/day) in percent	15 0	25 2			33 0			
		C	B	D	K	Ma	Mu	S
Total plasma fibrinogen (immunologically determined) in mg/100 ml before injection of ¹²⁵I fibrinogen	265	182	325	150	191	200	190	370
After 24 hours	273	220	333	158	240	208	226	370
After 3 days	273	253	360	188	273	226	194	416
After 7 days	273	234	355	168	260	226	194	468
After 12 days	273	235	334	200	290	234	208	460

was done at 15 and 30 min and at 1 6 12 and 24 hrs after injection later at daily intervals 1 5 ml aliquots of blood were heparinised with an un weighed lyophilised crystal of ammonium heparinate (ROCHE) Fibrinogen determinations were carried out by radial Immunodiffusion (9 11)

¹²⁵I fibrinogen determination 0 25 ml of plasma were incubated for 2 hrs at 37°C then for 24 hrs at 4 C with 0 2 ml monospecific anti human fibrinogen serum from the rabbit (8) The resulting immunoprecipitate was centrifuged at 15 000 g for 2 min and washed three times in buffered physiological saline The radioactivity of all the precipitates was measured at the end of each experiment with a Packard Auto-Gamma Spectrometer (Model 3575) The activity 30 min after application of the ¹²⁵I fibrinogen was taken as 100 The other measurements were expressed as ratios of this value after correction for zero emission

External body measurements For measurements over various organs a shielded Geiger Muller tube was used In this way background interference was reduced four fold as compared with a scintillation counter The detector tube was connected to a digital counter the assay time was preset according to the expected impulse rate The body measurements were taken in the following four positions

1 Over the heart Third intercostal space centred 3 cm from the sternal border
2 In the right axilla *Pointing exactly towards the* opposite axilla
3 Over the right thigh Over the diaphysis of the right femur and at 90 to this
4 Over the right temporal region On the midline between external orbita and external auditory meatus pointing exactly towards the opposite temporal region

Statistical calculations Arithmetical means were calculated for the ¹²⁵I fibrinogen activities measured between the third and twelfth day after injection After demonstration of highly significant differences between the fibrinogen activity curve characteristics in the adult the healthy prematures and the prematures with IRDS variance analyses were carried out on the IBM 7040 computer in the Dept of Mathematics University of Freiburg [1]

RESULTS

Fate of ¹²⁵I fibrinogen in adult

85μCi of ¹²⁵I-fibrinogen were injected intravenously and plasma disappearance rate was measured in the manner described above It can be seen (Fig 1, diagram 1) that after an initial rapid fall in concentration linear disappearance ensues at about 24 hours after injection (semi logarithmic scale) The half life is about 4 5 days or 108 hours corresponding to a catabolic rate of 15% per day This result compares well with recent results in the literature (1 4) whereas McFarlane (12) gives 7 hours as being the normal value Assuming th the steep fall in activity during the first day

[1] Our thanks are due to Dr Bloedhorn Institute of Medical Statistics and Documentation University of Freiburg

brinogen molecule thus leading to rapid catabolism and false results in half life determinations In order to exclude such an error the elimination of ^{1} I fibrinogen was determined in an adult as a control The half life of 4 5 days calculated corresponds to the recent data in the literature We conclude that the product used had b en little altered by ^{1} I tagging

Reference to the differing behaviour of fibrinogen *in vivo* in young and adult rabbits is mad by Atencio et al (2) These authors found a higher turnover rate in the young rabbits and explained this difference partly by the latively large extravascular fraction of total ibrinogen partly by a higher catabolic rate

Comparison of activity with the applied dose and body weight allows the assumption that he value 30 min after injection is representative for complete distribution and can be taken to be 100 Our results demonstrate a similarly increased extravascular fibrinogen fraction in premature infants as compared with the adult. Moreover either the influx into the extravascular compartment is slower or catabolism is increased during the first 2–3 days of life An accelerated elimination of adult fibrinogen by the newborn infant must be taken into account considering the existence of a specific foetal fibrinogen (14) This is made improbable however by the fact that activity over head and thigh rises concomitantly with the fall in plasma activity (Fig 3) These are areas with a relatively sparce vascularisation where the measured activity would presumably emit to a large degree from the extravascular compartment.

The interpretation of this result would be that $^{1\ 5}$I fibrinogen slowly diffuses from the intravascular compartment and accumulates in the lymph until equilibrium is reached after 2–3 days whereupon curves 4 and 5 (head and thigh Fig 3) run parallel to curve 1 (plasma)

In the body measurement shown in Fig 4 (IRDS) as compared with Fig 3 (normal premature) it is of particular interest that the activity over the h[illegible]t turning to run parallel to the other curves only after 24 hours It is likely that this is due to the typical fibrinogen deposition in the hyaline membranes of IRDS

Nothing can be said about half life and turnover during the first 2 days as the curves do not form a straight line (labile phase) Analysis of the curves seems to suggest a second e function but the scatter is too large for certainty It must be assumed that fibrinogen turnover is rapid in this early phase as well

From the third day on all three curves are straight (stable phase) Variance analysis proves highly significant differences in the catabolic rate in normal premature infants and those suffering from IRDS as compared with the adult (Fig 1) According to the half life of 2 8 days a turnover of 25 2° per day can be calculated for normal prematures Infants with IRDS recovering had 2 1 days and 33 turnover per day In the adult only 15°₀ of the fibrinogen pool is turned over The catabolic rate in surviving prematures with IRDS is one third higher than that in normal prematures but the difference is not significant. Neither infant dying of IRDS showed peculiarities during the first 3 days of life (Fig 2) On the third day after injection (third day of life) there was however very little remaining plasma ^{1} I fibrinogen activity (N I 7 6 ⁄ N II 5%) The first twin (N I) died of intraventricular haemorrhage The massive clot had the same ^{1} I fibrinogen activity/mg fibrinogen as a plasma sample with the same fibrinogen concentration from the second day of life It can be assumed that the main haemorrhage took place on this second day at a time when the clinical condition deteriorated with apnoea and symptoms of shock

In both groups examined the fibrinogen level rose during the first three days of life and then remained constant in spite of the high catabolic rate of ^{1} I fibrinogen This once again demonstrates the excellent regenerative capacity of even the premature newborn infant

There is no insufficiency in liver synthesis of fibrinog n even in the infants with severe

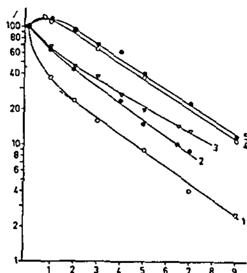


Fig 3 ^{125}I fibrinogen activity over various organs in a normal premature infant (D 1900 g) *1* Plasma *2* heart *3* right axilla *4* thigh *5* temporal area Abscissa Days after injection of ^{125}I fibrinogen and *days of life*

^{125}I-fibrinogen turnover in premature infants dying of IRDS

Male twins (N I and N II) were examined who died at the ages of 3 75 days and 3 0 days respectively The first twin had massive intra ventricular haemorrhage, the other had histologically verified intravascular fibrin clots in the vessels of the liver The rate of ^{125}I fibrinogen disappearance was unusually high in these two infants Premortal values were 7 6% (N I) and 5% (N II) of the original ^{125}I fibrinogen value (Fig 2)

Immunologically determined fibrinogen concentrations

The plasma fibrinogen values in the two groups of premature infants are shown in Table 1 It can be seen that a rise in concentration takes place in both groups during the first three days of life This conforms with results achieved earlier showing a steady rise in fibrinogen concentration during the first day of life (10)

Measurement of ^{125}I-fibrinogen activity over various organs in normal premature infants

A typical example is given in Fig 3 The characteristic sharp fall in the ^{125}I fibrinogen plasma level in the first two days is apparent The half-life is 2 3 days Activity over the heart and in the right axilla in which latter position mainly activity over the right lung is m as ured falls almost in a linear function when plotted semi logarithmically In contrast to this, activity rises over the temporal region and over the thigh to values above initial up to the 24th hour During the following days the curves continue almost parallel to that of ^{125}I fibrinogen in plasma

Measurement of ^{125}I fibrinogen activity over various organs in infants suffering from IRDS

Fig 4 shows a typical curve for a premature infant with IRDS Here again there is a rapid fall in ^{125}I fibrinogen activity during the first 2 to 3 days followed by a stable phase The half life is 2 0 days The values over the heart (2) and head (5) correspond to those found in normal premature infants (Fig 3) however there is no rise in activity over the thigh

Noticeably different from normal premature infants are the values over the right axilla (lung) A definite rise in activity takes place up to the 24th hour after injection

DISCUSSION

McFarlane (12) was able to show that over iodinisation can produce changes in the fi

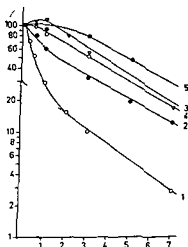


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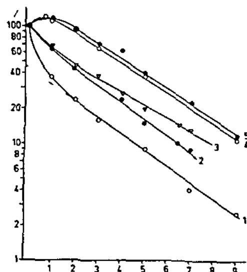


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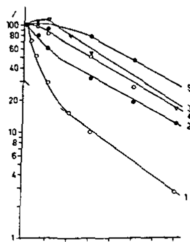


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IRDS Fibrinogen turnover in premature infants suffering from IRDS is, at least from the third day of life on, high enough to compensate for loss into the hyaline membranes and into intravascular clots This means that lack of one of the classical signs of intravascular coagulation in the adult, namely hypofibrinogenaemia, does not rule out i v C in IRDS The turnover in these infants is more than double that in adults, thus allowing even a rise in plasma fibrinogen concentration during the course of the disease

SUMMARY

Measurement of the elimination of small quantities of ^{1} I fibrinogen (5–15 μCi) demonstrates shortened half-life and higher catabolic rates in premature infants, and particularly in those infants suffering from IRDS compared with adults Turnover in the adult is 15%, in the premature 25% and in the premature with IRDS 33% per day

Equilibrium between intravascular and extravascular fibrinogen is not achieved before the third day of life Later elimination is linear There is a highly significant difference between ^{1} ^{1}I-fibrinogen catabolism in the adult and the premature infant with and without the IRDS between the third and twelfth day

Measurement of activity over various organs demonstrates that slow eflux of ^{1} I fibrinogen into the extravascular compartment causes a continuous fall in plasma activity during the first 2–3 days In premature infants with IRDS the activity over the lungs rises during the first day of life These results allow the conclusion that accelerated fibrinogen synthesis in infants with IRDS compensates for losses into the hyaline membranes and intravascular clots and even allows the physiological rise in plasma fibrinogen level during the first three days to take place

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RUBIDOMYCIN IN BLASTIC PHASES OF ACUTE PROMYELOCYTIC AND MYELOBLASTIC LEUKEMIA IN CHILDREN

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The fir t reports on rubidomycin and daunomycin effectiveness in acute leukemia in 1966 (9 13) were follow d by many studies on its action in leukemias (1 2 5 6 7 10) and tu mours (8 11 12 14) Only a few publica tions however deal with the use of rubidomycin in other than lymphoblastic forms of acute leukemia in children

CLINICAL MATERIAL AND METHODS

Rubidomycin therapy was introduced in our clinic in 1967 In 25 months 21 courses of treatment have been administered to 11 children with other than lymphoblastic forms of acute leukemia The diagnosis of acute leukemia was established on the basis of morphological and cytochemical criteria (peroxidase PAS)

The series includes 13 treatment courses in promyelocytic leukemia (4 cases) 5 courses in myeloblastic leukemia (4 cases) and single ca,es of erythroleukemia acute monoblastic leukemia and acute mye loblastic transformation of chronic granulocytic leuke mia The age of the patients ranged between 1 and 14 years

The blastic phases in which rubidomycin has been used are shown in Table 1

The relationship between the d sease period and rubidomycin therapy was as follows shortly after the diagnosis of acute leukemia 5 courses of treatment were administered Thereafter in the first and second halves of a year of illness 8 and 6 courses respectively were administered and 2 years after diagnosis two courses

Rubidomycin was usually given with prednisone in the dose 3 mg per kg body weight (13 courses) and less often alone During remission treatment with 6-mercaptopurine was introduced alternating with methotrexate The treatment was supplemented by low protein purine free diet (4) Rubidomycin was given in fast intravenous injections of 1 mg/kg body weight (mean dose 1 2 mg/kg) every second day (10 treatment courses) every 3 days (6 courses) or at longer intervals (5 courses)

RESULTS

The therapeutic results were assessed on the basis of the following criteria

Complete remission

I Hematologic criteria (*a*) peripheral blood disappearance of parablasts Hb ≥ 65 % erythrocytes ≥ 3 500 000/mm³ granulocytes ≥ 1 500/mm³ blood platelets ≥ 150 000/mm³ (*b*) bone marrow parablasts and blast cells count ≤ 10 % II Clinical criteria disapp ar ance of all clinical symptoms of leukemia and leukemic infiltrates accessible to examination

Partial remission

Separate decrease in the number of parablasts and blast cells in the bone marrow < 10 %

Beginning in 1969 stricter criteria were adopted (< 5 % parablasts and blast cells in myelograms) Earlier efforts had already been made to obtain more pronounced decrease of parablastosis in the bone marrow so that in 6 out of 11 remissions the bone marrow con tained less than 5 % parablasts and blast cells and in the remaining 5 cases percentages of these cells were less than 7 5 % The frequencies of remissions are shown in Table 2

Table 1 *Rubidomycin courses in different blastic phases of the disease*

Number of blastic phases	Number of treatment courses
I–II	14
III–IV	5
V–VI	2
Total	21

In promyelocytic leukemias, remissions were observed even in late relapses of the disease, as shown in Table 3

The favorable results in relapses may be attributed partly to early diagnosis and introduction of the rubidomycin therapy before overrunning of the bone marrow by parablasts

Promyelocytic leukemia in children is not infrequently characterized by tumours of the head (3) It has been observed that the reappearance of exophthalmos or a tumour in the cranium is very characteristic of relapse in children with this form of leukemia and that it is invariably accompanied by blastic proliferation in the bone marrow The bone marrow in relapses diagnosed in this way was not overrun completely by parablasts Cranial tumours were never observed in bone marrow remissions, which were always of short duration The rapid rate of appearance of relapses was the reason for prolonged hospitalization

In all cases the mean dose of rubidomycin per treatment course was low Remissions appeared on the average 10 days after the beginning of treatment In promyelocytic leukemia a greater number of relapses was treated so that the total dose of rubidomycin per child was larger (12 5 mg/kg body weight) than in other forms of acute leukemia (4 9 mg/kg)

Toxic symptoms In 8 out of 21 treatment courses no side effects were observed In the remaining treatment courses 26 harmful symptoms occurred including 19 pertaining to the blood Neutropenia was observed in 10 treatment courses In one half of courses a toxic neutropenia occurred alone, and less frequently it was encountered as a component of pancytopenia associated with thrombocytopenia, or combined only with anaemia Marked neutropenia appearing in the course of treatment (2) was considered to be an indication to change the administration of rubidomycin

Table 2 *Remissions in cases treated with rubidomycin*

Form of acute leukemia	Total number of treatment courses	Remissions total and partial	Percentage of total and partial remissions
Promyelocytic	13	8	61
Myeloblastic and others	8	3	37
Total	21	11	52 3

Table 3 *Number of remissions in successive blastic phases treated with rubidomycin*

Form of leukemia	Blastic phases					
	I–II		III–IV		V–VI	
	Treatment courses n	Remissions n	Treatment courses n	Remissions n	Treatment courses n	Remissions n
Promyelocytic	7	5	4	2	2	1
Myeloblastic and others	7	3	1	0	0	0
Total	14	8	5	2	2	1

Ulceration of the oral cavity was observed sporadically ECG abnormalities were some tim s difficult to interpret Three times the changes observed were attributed to the tox icity of rubidomycin One child in a resistant phase died suddenly from cardiovascular fail ure which seemed to be connected with rubidomycin therapy

DISCUSSION

Our obs rvations indicate that rubidomycin is effective in those forms of acute leukemia in children in which remissions are otherwise very rare This applies especially to promyelocytic leukemia which may be defined as hyper acute" because of its exceptionally rapid course Particular sensitivity of promyelocytic leukemia to rubidomycin therapy has been emphasised (1 2 15) The high percentage of remissions in promyelocytic leukemia including late re lapses may b attributed to earlier diagnosis The total dose of rubidomycin per treatment course was relatively low A larger number of cures was carried out in promyelocytic leu kemias during repeated blastic exacerbations so that the total dose of rubidomycin in this form per child (12 5 mg/kg body weight) was larger than used to be in the other forms of acute leukemia (4 9 mg/kg) in children

In a majority of cases prednisone treat ment was associated with the rubidomycin ther apy Combined therapy has been recommended by other authors (6 7) In our experience simultaneous administration of rubidomycin prednisone and low protein diet in acute lymphoblastic leukemia increases the percen tag of remissions In other morphological forms of the disease prednisone only modifies the intensity of harmful hematologic side ef fects of rubidomycin

It should b emphasised that treatment with rubidomycin of acute leukemia especially promyelocytic and myeloblastic which are char acterized by a tendency to neutropenia requires utmost caution and ought not to be schematic Flexibility of the intervals between injections is safer than daily administration of rubidomycin Before each injection the decision to continue the drug should be preceded by a detailed analysis of the patient s clinical state and blood picture Frequent myelograms are desirable

SUMMARY

Rubidomycin was administered in do es of 1 mg/kg body weight usually in combination with prednisone The best results were ob tained in promyelocytic leukemia Promyelocytic leukemia in children seems to be charac terized by sensitivity to rubidomycin even in late exacerbations

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NITROBLUE TETRAZOLIUM REDUCTION BY NEUTROPHILS OF NEWBORN INFANTS IN IN VITRO PHAGOCYTOSIS TEST

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The process of phagocytosis induces metabolic chan es in normal neutrophils Before the in tracellular killing of ingested organism takes place an increased proportion of glucose is metabolized via the hexose monophosphate shunt There is also a marked increment in oxygen consumption and hydrogen peroxide production (7) Such metabolic events are ac companied by a reduction of nitroblue tetra zolium (NBT) dye (1)

Baehner & Nathan (1) and Windhorst et al (13) using NBT and a combined phagocyto-sis histochemical test showed that during *in vitro* phagocytosis of latex particles the capacity of normal human neutrophils to reduce the dye was significantly increased Park et al (9) showed that a supravital method based on the *in vitro* reduction of NBT can be used to dif ferentiate certain types of bacterial infection from non bacterial illnesses During the course of a natural infection a large number of pe ripheral neutrophils will reduce NBT whereas a small proportion of neutrophils will reduce this dye without challenge The spontaneous reduction of NBT by neutrophils was found to be increased in phagocytes of term (5 10) and premature (3 4) newborn infants in ab-sence of bacterial infection thus resulting in a falsely positive NBT test

The purpose of this study was to determine the NBT reducing ability of term and pre mature infant, and adult leukocytes employing an *in vitro* phagocytosis system consisting of whole blood with *Pseudomonas aeruginosa* as test organism

MATERIAL AND METHODS

The study groups consisted of 17 clinically normal pre term infants (premature) of gestational age less than 37 weeks (11 12) from 1 to 12 days old (weight range 880–2 300 g) 6 pre term infants from 7 to 18 days old (weight range 980–2 180 g) with known bac terial diseases (skin abscesses with bacteriemia sep-ticemia bacterial meningitis and pneumonia) 14 ap-parently healthy term infants from 12 hours to 23 days old (weight range 2 540–4 100 g) and 7 adults

Venous blood was drawn under aseptic condition transferred into disposabl capped plastic tube and 50 units of heparin (Boots) per ml of blood were added

The histochemical NBT reduction test was carried out using the method of Park et al (9) Approximately 0 1 ml of blood was gently mixed with an equal amount of 0 2% NBT (Sigma) solution and 0 15 M phosphate buffered saline solution pH 7 2 The mix ture was incubated at 37 C for 15 min and sub-sequently kept at room temperature for an addi tional 15 min Coverslip smears were prepared air dried and then counterstained with Wright stain (BDH) All slides coded and examined under the microscope with oil immersion were read by the same investigator (P C) who did not know the source of the cells The percentage of neutrophils containing a large black deposit of formazan (reduced NBT) were classified NBT positive NBT tests were performed in all the blood samples a few minutes after the drawing of blood and before adding bacteria

The phagocytosis experiments were carried out in plastic tubes by adding bacteria to who e heparinized blood *Pseudomonas aeruginosa* grown in Lowbury selective medium served as the test organism and was obtained from the stock culture collection at the

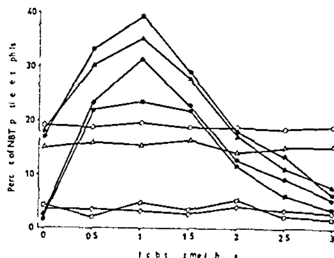


Fig 1 NBT reducing activity by leukocytes in phagocytosis experiments Blood from premature (closed circles) and term (closed triangles) infants premature infants with bacterial infections (closed squares) and adults (closed lozenges) incubated with *Pseudomonas aeruginosa* Blood from premature (open circles) and term (open triangles) infants premature infants with bacterial infections (open squares) and adults (open lozenges) without adding bacteria

Pediatric Clinic (2) Bacterial suspensions were made from 24 hours old cultures washed twice with Hanks balanced salt solution and resuspended to a known concentration in saline solution The inocula in the volume of 0 05 ml contained approximately 10 [8] organisms The number of test organisms in the original suspension was determined by plate count method on blood agar The mixture consisting in 2 ml blood and bacteria was then incubated at 37 C in roller drum at 1/8 rpm At 30 min intervals blood samples were removed and tested for the *NBT* reducing activity of leukocytes

Control NBT reduction activity in blood samples without additional bacteria obtained from 9 healthy premature 8 term infants 6 premature infants with bacterial diseases and 7 adults was also determined

All the blood samples including that of the controls from which bacteria were omitted were treated in the same way A total of 74 blood samples obtained from 44 subjects were studied The results of the NBT tests in the bloods of the premature full term infants and adults were compared with those of controls

RESULTS

Histochemical NBT tests were carried out on a series of premature infants term infants and adults leukocytes at rest and during phagocytosis The results of the NBT reducing activity by neutrophils from newborn infants and adults in *in vitro* phagocytosis tests are presented in Fig 1 The percentage of NBT positive neutrophils in various groups are summarized in Table 1

A significantly greater percentage of neutrophils incubated with *Ps aeruginosa* reduces NBT if compared with the neutrophils of controls without additional bacteria ($p<0\ 01$) The histochemical test determinations on blood samples taken at intervals after adding bacteria showed progressive increasing of NBT positive neutrophils The mean percentage in premature and term infants was, at first reading, 17 6 (S D ±6 78) and 17 7 (S D ±10 3) respectively, then it rapidly rose reaching after 0 5 hour of incubation with *Ps aeruginosa* 33 2 (S D = ±14 9) and 30 3 (S D = ±17) respectively The greatest number of NBT positive leukocytes was found after 1 hour of incubation with a mean percentage of 39 4 (S D = ±12 9) in premature infants and 35 5 (S D = ±16 5) in term infants In both groups the proportion of NBT positive neutrophils then progressively decreased to 6 5 % (S D = ±3 4) and 8 1 % (S D = ±6 9) after 3 hours of incubation The percentage of NBT positive neutrophils in premature infants with bacterial diseases and adults was, at first reading 2 1 (S D = ±1 4) and 2 7 (S D = ±1 7) respectively These mean values progressively increased reaching after 1 hour of incubation, 23 7 % (S D = ±13 9) in premature infants and 31 5 % (S D = ±20 2) in adults In both groups the proportion of NBT positive cells then decreased to 6 2 % (S D = ±2) and 3 5 % (S D = ±1 3) respectively after 3 hours of incubation

In the uninoculated blood controls the initial values of NBT positive neutrophils did not vary significantly with the incubation time The mean percentage of NBT positive neutrophils ranged between 18 6 (S D = ±11 2) and 19 7 (S D = ±10 6) in premature infants 3 2 (S D = ±1 8) and 5 1 (S D = ±2 9) in premature infants with bacterial infections, 14 1 (S D = ±6 9) and 16 6 (S D = ±12 1) in term infants and from 3 (S D = ±1 1) to 4 (S D = ±2 3) in adults

Table 1 *NBT reduction by neutrophils of newborn infants in* in vitro *phagocytosis test*

Age groups	No		Percent NBT positive neutrophils at various time intervals of incubation (in hours) 0	0 5	1	1 5	2	2 5	3
Bloods incubated with *Ps aeruginosa*									
Premature infants	17		17 6	33 2	39 4	28 8	17 8	13 5	6 5
		S D ±	6 7	14 9	12 9	8 3	8 4	6 4	3 4
Premature infants with bacterial infections	6		2 1	22	23 7	22	12 7	9 5	6 2
		S D ±	1 4	11 6	13 9	9 9	3 5	5	2
Term infants	14		17 7	30 3	35 5	28 7	17 5	11 4	8 1
		S D ±	10 3	17	16 5	15 5	13	10 5	6 9
Adults	7		2 7	23 5	31 5	22 5	12	6 2	3 5
		S D ±	1 7	17 9	20 2	5 9	5 1	1 2	1 3
Uninoculated blood controls									
Premature infants	9		18 6	18 8	19 7	18 8	18 8	18 5	19 1
		S D ±	11 2	10 9	10 6	10 9	5 4	11 4	11 6
Premature infants with bacterial infections	6		3 2	2	4 7	3 1	5 1	2 2	2 1
		S D ±	1 8	1 5	1 9	2.1	2 9	1 8	1
Term infants	8		15 1	16 1	15 5	16 6	14 1	15	15 6
		S D ±	7 2	9 9	7 8	12 1	6 9	7 9	11 8
Adults	7		3	3 5	3 1	2 9	4	3 2	2 8
		S D ±	1 1	1 9	2 4	1 7	2 3	1 8	1 9

DISCUSSION

The results of the histochemical NBT test in *in vitro* phagocytosis experiments showed the expected rise of NBT reduction by phagocytosing neonatal and adult leukocytes When incubated with *Ps aeruginosa* leukocytes from newborn infants and adults reduce NBT dye to a statistically significant greater extent than do resting control leukocytes without additional bacteria These data confirm the results of Baehner & Nathan (1) and Windhorst et al (13) who reported that during phagocytosis the NBT reducing activity of normal human leukocytes was considerably increased

In our studies the NBT test determinations on blood samples taken at 30 min intervals after adding bacteria showed a progressive increase of NBT positive neutrophils with the maximum rate of reduction after 1 hour of incubation at 37 C in all groups

The mean percentage was at first reading premature infants 17 6 premature infants with bacterial infections 2 1 term infants 17 7 and adults 2 7 After 1 hour of incubation it was 39 4 23 7 35 5 and 31.5 respectively These mean values then decreased and reached their lowest degree after 3 hours of incubation

On the contrary in the uninoculated control groups no definite increase of spontaneous NBT reduction activity was observed by resting neutrophils at the same incubation time even in the group of premature infants with documented bacterial infections The mean percentage of NBT positive leukocytes did not vary significantly from the initial values

We previously reported (3 4) that leukocytes obtained from healthy premature and term infants reduce NBT to a greater extent than do neutrophils of premature infants with acute bacterial infections The worsening of the infection is associated in these subjects with a decreasing of NBT reduction On the other hand improvement and recovery were associated with an increase in the proportion of NBT positive neutrophils The reason for the decreased NBT reduction activity in premature infants in natural infections but not *in vitro* phagocytosis experiments remains to be explained Preliminary study suggests that newborn leukocytes consume twice as much oxy

gen as control cells (10), but specific enzyme or enzymes involved in the reaction have certainly not yet been identified (6)

On the other hand in *in vitro* phagocytosis experiments, here reported, the values of the first reading agree with the already observed NBT reduction activity of the circulating leukocytes from neonates, tested without further *in vitro* challenge, particularly for the premature infants with bacterial infection Although these findings may indicate that neutrophils are undergoing *in vivo* metabolic changes similar to those associated with *in vitro* phagocytosis, the incubation with *Ps aeruginosa* did not reveal a decrease of NBT reduction activity by leukocytes of premature infants

It may be concluded that the reduction of NBT by neutrophils from neonates and adults significantly increases when they are incubated *in vitro* with *Ps aeruginosa* with the maximum rate of reduction after 1 hour of incubation Furthermore a difference in behaviour between neutrophils of premature infants with bacterial infection during *in vitro* phagocytosis experiments and those phagocytosing *in vivo* during a natural acute infection of bacterial origin is suggested by the different rate of NBT reduction

SUMMARY

Venous blood from healthy premature infants, premature infants with known bacterial infections, normal term infants and adults was incubated *in vitro* with *Pseudomonas aeruginosa* At 30 min intervals, blood samples were tested for the nitroblue tetrazolium (NBT) reducing activity of neutrophils The results of the histochemical NBT test were compared with those of the blood controls from which bacteria were omitted

The stimulation of neutrophils with *Ps aeruginosa* induces a considerable increase of NBT reduction in all 4 groups of subjects examined The maximum rate of NBT reduction was observed after 1 hour of incubation with the test organism, and reached its lowest degree after 3 hours By contrast the NBT reduction by unstimulated neutrophils of the same subjects remain unchanged during a 3 hour period of incubation

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DERMATOGLYPHICS AND THE SIMIAN CREASE IN INFANTS OF LOW BIRTH WEIGHT

A Pilot Study

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The dermal ridges are in their complete and permanent form by the end of the fourth month of intra uterine life (8) and the palmar creases are formed even earlier (6) Although dermatoglyphic features are primarily under genetic control the infant s developing dermatoglyphics may be altered by environmental factors acting on the fetus including maternal rubella (2) and thalidomide (8)

It is now accepted that many low birth weight (LBW) infants are born after a normal gestation period i e "Small for dates These undergrown infants are probably associated with various causes including fetal malnutri tion and hypoplastic growth disturbances (11)

This pilot study sought dermatoglyphic find ings in a group of LBW infants on the premise that intra uterine disturbances leading to the ultimate birth of a Small for-dates infant might also cause unusual dermatoglyphics This study may be regarded as an extension of the work of Davies (5) who found an increased incidence of prematurity among neonates with a single transverse palmar crease

MATERIAL AND METHODS

73 neonates aged 1–49 days, weighing less than 2 000 g at birth 33 males and 40 females, Jews and Arabs formed the study (LBW) group They included 3 pairs of twins and a further 11 single members of a twinship All were apparently without malforma tions They were examined in the premature units of the Rambam Hospital Haifa the Central Emek Hospital Afule the WIZO Babies Home Tel Aviv and the Kirya Maternity Hospital, Tel Aviv

Both hands were examined by direct inspection with a magnifying lens under appropriate illumina tion The following frequencies of features were ana lysed

Simian crease (simian line palmar crease)
- typical
- transitional

Interdigital patterns
High axial triradius
Hypothenar patterns
Thenar patterns
Finger patterns (loops whorls arches)

The criteria used for defining the dermatoglyphic features are according to those in the Memorandum on Dermatoglyphic Nomenclature" (9) Axial triradius was defined as high when its position was t t or t The schematic forms of typical and transitional forms of the simian crease as defined above are shown in Fig 1 (12) "Typical simian crease" (Fig 1 2) was recorded when there was a complete fusion of the usual two transverse palmar creases into one uninterrupted transverse line "Transitional" forms of simian crease (Figs 1 3 and 1 4) were recorded when the fusion of the 2 transverse lines was not comp ete or when they were clearly connected Fig 1 1 shows the normal form of palmar creases

Two control groups were examined viz.

1 100 normal neonates by direct inspection of the hands for dermatoglyphic features and simian crease
2 979 school children attending ordinary Haifa schools, by direct inspection of the palms for simian crease

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Fig 1 Forms of Simian line

RESULTS

The findings in the LBW infants and the control groups are summarised in Table 1

A higher frequency of simian crease was found among the LBW group The difference in the total incidence of simian crease (typical and transitional) between the LBW group and the control neonate group was significant at the 5 % level For each type of simian crease, there was a significantly higher incidence of simian crease in the LBW group as compared with the large group of school children controls ($p < 0\ 05$ for each type and $p < 0\ 005$ for the total frequency of simian crease)

There was no significant difference in the frequency in dermatoglyphic features studied between the LBW group and the control neonate group

Table 1 *Incidence ( ) of Simian crease and dermatoglyphic features in 73 low birth weight neonates and control groups*

100 normal neonates and 979 school children

Pattern present	73 LBW neonates	Control groups: 100 Normal neonates	979 School children
Simian crease			
Typical	5 4	1 0	2 0*
Transitional	10 9	5 0	4 7*
Total	16 3	6 0*	6 7**
Dermatoglyphic features			
High axial triradius	6 8	7 0	
Palmar patterns			
–hypothenar	16 5	15 0	
–thenar	1 3	1 0	
Interdigital patterns			
I	0 0	0 0	
II	1 3	4 0	
III	41 0	36 0	
IV	23 2	29 0	

Differences between LBW group and control group statistically significant at 5 level * and at 1 level **

DISCUSSION

Many clinical disorders are known to be associated with an increased incidence of simian crease Some authors have included transitional forms within their studies (2, 10) whereas others included only typical forms (1 5)

Our previous study (4) described pedigrees showing the occurrence of the various forms of simian crease *within the same family* This suggests that transitional forms are different expressions of the same entity It seems justified therefore to group transitional and typical forms together in studies of simian crease frequency

Since a significant number of LBW infants are small for gestation the LBW group included Small-for dates infants The percentage of Small for-dates infants within the LBW group has been estimated as 31 % (3) or even as high as a half (7) Early intra uterine disturbances responsible for the birth of a Small for dates infant might also lead to unusual dermatoglyphic features in these infants

Davies & Smallpiece (6) reported an increased incidence of prematurity among neonates with a typical simian crease This association between simian crease and LBW neonates is confirmed by our findings

No attempt was made to assess the duration of gestation in these 73 newborns It would seem worthwhile to study the dermatoglyphic features of LBW infants with a known short gestation period others with a known normal gestation period and relatives of these two groups of children

SUMMARY

Dermatoglyphic features and simian crease incidence were studied in 73 neonates with a birth weight under 2 000 g There was a higher frequency of simian crease typical and transitional types as compared with two control groups of 100 normal neonates and 929 school

children There was no significant difference in the incidence of dermatoglyphic features between the low birth weight and the control neonates

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CASE REPORT

HEPATIC INVOLVEMENT IN THE COURSE OF ACQUIRED TOXOPLASMOSIS

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The characteristic clinical manifestations of congenital toxoplasmosis are found in the central nervous system, while acquired toxoplasmosis most frequently presents as generalized lymphadenopathy Reports of visceral or abdominal toxoplasmosis are not numerous, indicating that this form is uncommon According to Desmonts (2), splenomegaly is seldom found in children with acquired toxoplasmosis

We present here the case history of a child suffering from hepatosplenomegaly and lymphadenopathy

CLINICAL HISTORY AND FINDINGS

The patient J S a 5 year old girl lived in the environs of Prague Neither the family nor the personal history revealed anything relevant to toxoplasma infection Eleven days before our patient fell ill her three year old brother contracted measles with a typical clinical course Apparently he became infected in a public nursery school (crèche) where measles epidemic had been attacking a number of children The disease of our patient started on October 29 with prodromal catarrhal symptoms and appearance of typical maculopapular eruption on the skin At the same time (November 1–2) she complained of abdominal pain vomited repeatedly and so was referred to the Clinic of Infectious Diseases on November 2 1966 On admission an acute abdomen was excluded The typical appearances of measles were seen but nothing else The liver spleen and lymph nodes were all normal During the next three weeks enlargement of liver and spleen developed together with a marked increase in serum glutamic oxalacetic acid transaminase (SGOT = 5 4 μmol/ml) and serum glutamic pyruvic acid transaminase (SGPT = 10 0 μmol/ml) Eosinophilia was found in the peripheral blood Throat culture showed the growth of β haemolytic streptococci and the antistreptolysin titre was elevated

Since these changes do not occur in measles the patient was transferred to the Paediatric Clinic of the Institute for Post Graduate Medical Education in Prague on November 25 1966 Rheumatic disease was suspected The levels of serum transaminases were found to fall but the hepatosplenomegaly and the eosinophilia persisted A low titre complement fixation test (CFT) did not suggest acute toxoplasmosis and the patient was discharged on December 7 1966

At follow up examinations the temperatures were normal but weakness and fatigue were pronounced A second CFT showed a titre of 80 and so she was admitted to the Clinic of Infectious Diseases on January 13 1967 where she remained until February 8 1967 On admission the liver was palpable 4 cm below the right costal margin and the spleen was 3 cm below the left costal margin The lymph nodes were enlarged in both posterior cervical chains in both axillae and in both inguinal regions They varied in size from peas to cherries They were tender smooth of uniform consistency well defined and mobile They were covered by normal skin and were not painful on pressure

While in hospital the temperature never rose above 37 6 C Chest and skull X ray showed no pathological changes Ophthalmoscopic and electrocardiographic examinations revealed no abnormalities There was no skin rash and no jaundice

Laboratory investigations

Erythrocyte and leucocyte counts were within normal limits (3 2–4 2 million and 4 700–10 200/mm respectively) haemoglobin 70 % colour index 0 9 In differential counts the highest lymphocyte value was 57% and the highest eosinophile was 16% (Fig 1)

Table 1 *Liver function tests and serum transaminase activity*[a]

Date	Total bilirubin (mg/100 ml)	Thymol turbidity units	SGOT[b] (μmol/1 ml)	SGPT[c] (μmol/1 ml)	Serum Fe (μg/100 ml)
Nov 4 1966	0 5	4 9	5 4	10 0	
Nov 10 1966			1 4	1 7	
Nov 22, 1966			1 1	0 8	60
Dec 1 1966	0 4	2.4	1 1	0 4	
Jan. 16 1967		0 7	0 5	0 3	

[a] Determinated by colorimetry based on the principles of Reitman & Frankel s method (*Am J Clin Path* 28 56 1957)
[b] Normal range of activity is 0 35 to 0 95 μmol/ml
[c] Normal range of activity is 0 20 to 0 60 μmol/ml

No atypical lymphoid monocytes of the Downey type (glandular fever cells) were seen Sedimentation rate (January 16 1967) was 12/18 mm (Westergren) Results of the liver function tests are recorded in Table 1

Parasitological examination of faeces was repeated ten times and always yielded negative results Examinations for brucellosis listeriosis leptospiroses and tularemia were negative The Paul Bunnell test serologic tests for syphilis and Dubos-Middlebrook test were negative and skin tests for tuberculosis were weakly positive Urine contained neither protein nor bile pigments

Serological data Toxoplasma antibody tests were done 17 times over a period *of 4 years In three of* these the titre was *sufficiently high to indicate re* cently acquired and active *toxoplasmosis The first* CFT titre was 10 the second 80 and the highest 320 The indirect fluorescent *antibody test (IFAT) titres* paralleled the CFT but at a higher level The highest IFAT titre was 1 80 The highest titre of the Sabin Feldman dye test was 4 000 During convalescence and afterwards the antibody titres fell as shown in *Fig 1* The technical details and interpretation of our serological procedures have already been recorded (7)

Skin test with our heat treated toxoplasmin (6) revealed a strongly positive reaction after 48 hours (erythema 35 30 mm 3+ induration 25 × 25 mm 3+)

An enlarged lymph node was removed from the left groin on January 17 1967 The capsule was markedly fibrotic and the peripheral lymph sinuses were packed with lymphoid cells Proliferative epithelioid cells of the reticulum were scattered diffusely throughout the periphery of the node The germinal centres of the follicles were enlarged active and contained many mitotic figures (Dr Viklicky Dept of Pathology Inst for Postgr Med Ed)

Mice inoculated with a suspension of the patient s lymph node developed toxoplasma antibodies in the first passage However toxoplasma could not be demonstrated in this or in subsequent passages.

Treatment

The child was given anti toxoplasma treatment and then discharged in a good general condition afebrile and symptomless The liver and spleen decreased to 1.5 cm below the costal margin The lymph nodes decreased to normal

Subsequently the eosinophilia recurred and the raised CF titres persisted and so the child was given another course of treatment during a further stay at our clinic (June 15–30 1967) In both courses a triple combination of pyrimethamine (Daraprim Burroughs Wellcome England) sulfanilamido-methylpyrimidine (Sulfamethoxydin Spofa Czechoslovakia) and spiramycin (Rovamycine Specia, France) was given The total doses in these two courses were Daraprim 120 mg and 80 mg Sulfamethoxydin 3 100 mg and 3 000 mg and Rovamycin 10 g and 7 5 g respectively

During the two convalescence periods and since the patient has been repeatedly examined The child appears to be well The liver spleen and lymph nodes are within normal limits Liver function tests are normal and toxoplasma antibody titres are falling

Epidemiological enquiries

Small domestic animals such as fowl and rabbits with which the child came into contact but not cats were kept in the patient s household In the spring 1967 one of the rabbits died but the cause was not ascertained

DISCUSSION

Splenomegaly protracted fever and protozoa like bodies (Toxoplasma pyrogenes) in the blood and spleen were reported by Castellani as early as in 1914 Kabelitz (9 10) distinguishes two abdominal forms of postnatally acquired toxoplasmosis firstly primary liver toxoplasmosis with transient initial enterocolitis without either involvement of peripheral lymph nodes or lymphocytosis and secondly primary cervico-nuchal or generalized lymphadenitis with lymphocytosis In the latter liver involvement represents a late complication In both

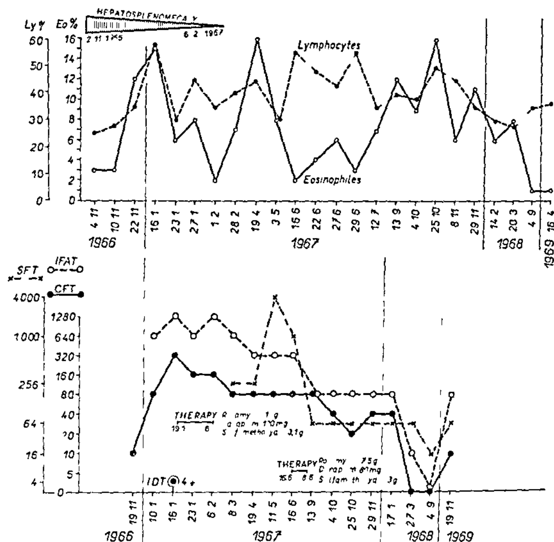


Fig 1 Acquired toxoplasmosis with hepatosplenomegaly and eosinophilia in a 5 year-old girl Toxoplasma antibody tests were done over a period of 4 years

groups a tender enlarged liver, mild enlargement of the spleen subicterus and an increased bilirubin level occur Geyer (4) describes a series of adult patients with cervical lymphadenopathy and simultaneous liver involvement In a subsequent report (5) Geyer describes the symptomatology of liver damage and the results of liver biopsies and also points out a possible relationship between toxoplasmosis and chronic liver damage Liver involvement in patients with generalized toxoplasmosis has been recently reviewed by Theologides & Kennedy (23) Vischer et al (25) reported two cases of a disease resembling viral hepatitis associated with high titre of toxoplasma antibodies as well as the demonstration of parasites in sections of liver biopsy Diamant Berger (3) Umdenstock & Raymond (24), Joseph et al (8) draw attention to abdominal symptoms in mesenteric lymphadenitis due to toxoplasma infection

The pattern of serum transaminase activity in a newborn infant with jaundice due to congenital toxoplasmic hepatitis was described by Kove et al (11)

Silver & Dixon (21) demonstrated a marked eosinophilia of the peripheral blood and bone marrow in a 6 week old infant with congenital toxoplasmosis Lelong et al (13) reported eosinophilia in 6 cases out of 227 patients with acquired toxoplasmosis According to Piquet et al (15) eosinophilia occurs more frequently in children than in adults We were unable to find a cause for our patient s eosinophilia other

than toxoplasmosis The recurrence of her eosinophilia during convalescence did not correspond with either the dynamics of her antibodies or with her clinical condition.

The histological features in glandular toxoplasmosis are not pathognomic but are characteristic and well known from the reports of many authors (16 17 18 19 22) The most frequent changes described by them are filling of lymph sinuses with proliferative reticulum cells proliferation of epithelioid cells either diffusely scattered or in clusters follicular hyperplasia with large germinal centers and basophilic granules (nuclear debris) situated either extra or intra-cellularly Demonstration of parasites is rare

In our patient we found hepatosplenomegaly with evidence for liver damage pluriglandular lymphadenopathy and eosinophilia. Rising antibody titres indicated the presence of toxoplasma infection. It could be questioned whether measles toxoplasma infection or both were the cause of the actual symptoms from lymph nodes liver spleen and peripheral blood In this connection the possible synergism of rubeola *virus* and toxoplasma parasite should be mentioned It is well known among veterinarians that concurrent distemper is one of determining factors *in the* causal genesis of toxoplasma lesions in dogs Koestner & Cole (quoted by Sum et al 20) pointed out stress factors such as distemper which had been found to be responsible for activation of latent toxoplasmosis Rubeola virus is antigenically similar to distemper virus (26) It can be believed that our patient acquired toxoplasmosis in a state weakened by concurrent or antecedent measles There were no signs suggesting an activation of congenital toxoplasmosis The source of infection may have been rabbits which in this country are almost all infected (12) A similar report of a child with a febrile illness clinical evidence of pneumonia generalized lymphadenopathy hepatosplenomegaly with abnormal liver function tests and eosinophilia was made by Neumann et al (14)

As may be seen either in the literature or in our report the symptomatology of abdominal toxoplasmosis is not specific and can imitate other diseases Subjective complaints (abdominal pain) and objective findings (hepatosplenomegaly changes in liver tests) may be caused by many agents of disease It is necessary to exclude appendicitis viral hepatitis infectious mononucleosis other causes of mesenteric lymphadenitis and intestinal parasites in children. The results of diagnostic tests for toxoplasmosis are of paramount importance It is not yet clear to what extent if any the activity of the infection can be related to eosinophilia In our patient the eosinophilia persisted longer than the clinical signs and high toxoplasma antibody titres

In conclusion we recommend that the possibility of toxoplasmosis should be considered in children with vague abdominal complaints and in those with hepatosplenomegaly

SUMMARY

The authors report hepatosplenomegaly in a 5 year old girl together with pluriglandular lymphadenopathy hepatic involvement and eosinophilia Antibody tests for toxoplasmosis revealed high titres The condition improved after treatment with toxoplasmacidal drugs

ACKNOWLEDGEMENT

The authors wish to express their thanks to Mrs D Princová for performing the CF tests and to Dr J Hubner for performing the dye tests

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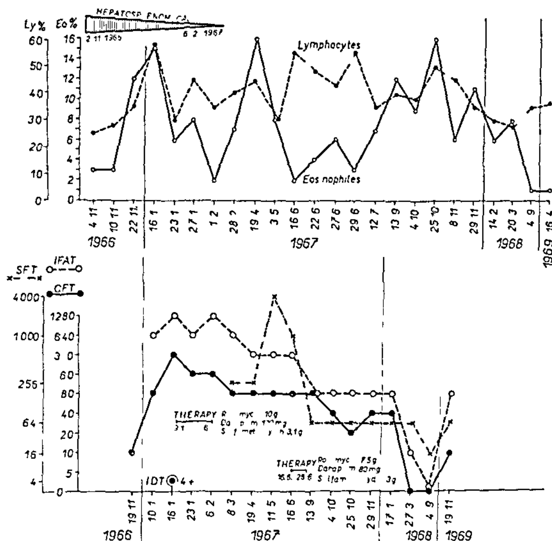


Fig. 1. Acquired toxoplasmosis with hepatosplenomegaly and eosinophilia in a 5 year old girl. Toxoplasma antibody tests were done over a period of 4 years.

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In this issue of Acta Paediat Scand Kouba Jira and Zitova report on a case of toxoplasmosis and measles with appearance of hepato-splenomegalia and pronounced eosinophilia symptoms usually not seen in single infections with either one of the two agents It should be noticed in this connection that measles virus is biologically closely related to distemper virus

In searching for a mechanism responsible for the possible synergistic effect of measles virus and Toxoplasma the following circumstances should be considered

Toxoplasmosis is accompanied by proliferation of phagocytizing reticulo-endothelial and large lymphoid cells both probably target cells in early stages of measles infection

There is good evidence that in toxoplasmosis as well as in measles local antigen antibody reactions occur In toxoplasmosis also circulating immune complexes appear It is conceivable that simultaneous formation of immune complexes locally in Arthus reactions and in the circulation may aggravate the inflammatory manifestations in spleen and liver sufficiently to produce symptoms

Finally both infections are accompanied by lymphocytopenia which probably explains the reduction in immunological reactivity particularly cell mediated immunity

The effect of double infection of lymphoreticular tissue is so far very little studied The case presented by Kouba and coworkers indicates that this etiology sometimes is of clinical significance It seems reasonable to assume that in the course of a long lasting infection like toxoplasmosis a patient may contract also a systemic virosis In cases with unusual symptoms from the RES the possibility of a double infection should be kept in mind

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Key words Hepatosplenomegaly lymphadenopathy toxoplasmosis

The Editors have asked dr Gunnel Huldt to comment on the Case Report by Kouba et al

Like most other parasites Toxoplasma gives rise to long lasting infections It can be demonstrated that the primary multiplication of the parasite takes place in reticuloendothelial and lymphatic tissue Pronounced reactions from such tissue is one of the main characteristics of toxoplasmosis Systematic studies on the duration of RES reactions in this infection are lacking, however

A number of agents mainly of viral origin attack and cause reactions from RES Double infections with Toxoplasma and one of these agents may act synergistically and give severe symptoms sometimes not recognizable in either one of the two infections involved

Studies in progress indicate that such synergistic effect can be demonstrated in mice experimentally infected with Toxoplasma and Moloney virus It is also well known among veterinarians that distemper in dogs runs a more severe course if the disease is complicated by infection also with Toxoplasma Necroses in

the liver have been demonstrated in such cases but are usually not found neither in toxoplasmosis nor in distemper in dogs

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REVIEW ARTICLE

LACTOSE INTOLERANCE AND PROTEIN MALNUTRITION

A DAHLQVIST and B LINDQUIST

From the Research Department J E Block and the Paediatric Department University Hospital Lund Sweden

Milk is undoubtedly an extremely valuable source of essential nutrients, especially protein The milk from most mammalian species, including man and cow, contains considerable amounts of a specific disaccharide, namely lactose This disaccharide seems to stimulate the absorption of calcium in the small intestine, but to our knowledge no other physiological effect of lactose making it superior to e g glucose as a nutrient, has been demonstrated In the small intestine lactose is hydrolyzed into glucose and galactose by small intestinal lactase These two monosaccharides are absorbed into the blood stream by a common active transport mechanism and the galactose is rapidly converted into glucose by the liver

During the last decade we have learnt that in some subjects the intestinal lactase activity is low or absent The administration of lactose to adult subjects with lactase deficiency often leads to abdominal discomfort and diarrhoea In infants with lactase deficiency more drastic symptoms will occur with watery diarrhoea which often leads to dehydration and severe malnutrition Continued milk feeding may in such infants lead to death Since the infants often have fever the symptoms may be mistaken as signs of intestinal infection

Lactase deficiency may thus severely interfere with the use of milk as a nutrient both in infants and adults and since some forms of lactase deficiency are very common as we shall see below, this enzyme deficiency has to be taken into account when milk is used as treatment for patients with different degrees of protein malnutrition Protein malnutrition as such may also lead to lactase deficiency and in this way interfere with the intestinal hydrolysis of lactose We will first describe the methods for diagnosis and the pathophysiology of lactose intolerance, and then the pathogenesis of the different forms of lactose intolerance Finally we will discuss the therapeutic possibilities

Methods of evaluating the intestinal disaccharidase activities

There are two important methods available namely oral tolerance tests and in vitro assay of the disaccharidase activities in biopsy specimens of the jejunal mucosa In infants analysis of sugars in faeces can also be of value for the diagnosis but in adults this analysis is of no value because unabsorbed sugar is consumed by the bacteria in the large intestine

(a) *Oral tolerance tests* After oral administration of any of the common dietary disaccharides (dose 2 g/kg body weight or 30–50 g/m body surface) normal subjects will show a blood glucose peak with a maximum after 30–60 min an increase of more than 25 mg/100 ml above the fasting level In a patient who is intolerant to the disaccharide in question the blood glucose curve will be flat (Fig 1) and the patient will get diarrhoea A control tolerance test with the corresponding mono-

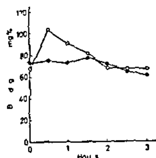


Fig 1 Blood glucose curve during a lactose tolerance test (●) in a 2 ½ year old boy with (congenital) lactose malabsorption The curve is flat Control tolerance test with a mixture of glucose and galactose (○) shows normal absorption of the monosaccharides ([illegible])

Table I *Disaccharidase activities in homogenized biopsies of human small intestinal mucosa from one control group of North Americans and one group with lactose intolerance (20 24)*

One unit of disaccharidase is the activity hydrolyzing 1 μmole of substrate per min at 37 C at pH 6 0 The activity has been calculated as units per g of protein

	Units per g protein			
	Control group (n = 27)		Lactose intolerant group (n = 12)	
	Mean	Range	Mean	Range
Maltase	266	111–470	234	94–505
Sucrase	87	26–138	77	19–194
Isomaltase	97	21–183	83	25–202
Lactase	44	9–98	2	0–6

saccharides is used to demonstrate that the monosaccharide transport is unimpaired (Fig 1)

(b) *Disaccharidase activity assay in biopsy specimens* For this examination a piece of small intestinal mucosa (usually taken at the duodeno-jejunal flexure) is obtained by peroral

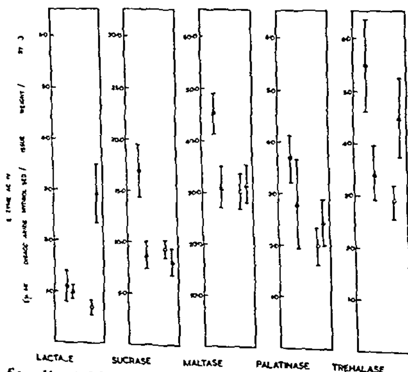


Fig 2 Mean (± S D of the mean) of disaccharidase levels in jejunal biopsy specimens Results for Baganda (●) and Bahutu (Rwanda) (○) children who had previously had kwashiorkor and for Baganda (▲) and Bahutu (Rwanda) (△) controls The Bahutu are a mixed group of Bantu and Hamitic (Batutsi) background and they normally retain high activity of intestinal lactase throughout life The Baganda tribe has a very high incidence of (racial) lactase deficiency after the age of 4 years From Cook & Lee (16)

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Table 3 *Disaccharidase activities in intestinal mucosa from 33 kwashiorkor patients (35) compared with normal values obtained from children (10) and adults (24)*

	Units/g protein (mean and range)		
		Normal values	
	Kwashiorkor patients	I children	II adults
Lactase	7 (1–17)	49 (14–132)	44 (9–98)
Sucrase	35 (5–71)	95 (32–228)	87 (26–138)
Isomaltase	51 (3–118)	89 (31–177)	97 (21–183)
Maltase	100 (0–?26)	260 (83–615)	266 (111–4 0)

the carbohydrate composition of the food Dietary lactose has no influence whatsoever on the intestinal lactase activity Sucrose (and fructose) exert some influence on the sucrase and maltase activities but this influence is rather small and does not seem to be the major regulating factor (36) As we shall see below some humans retain the high lactase activity through life but there are also large groups of humans which lose their lactase after the first years of life In man as in animals dietary lactose does not influence the lactase activity of the mucosa (36)

Different forms of lactase deficiency

There seems to exist three different forms of la tase deficiency all probably differing in their pathogenesis

(a) *Congenital hereditary lactase deficiency* The intestinal lactase is missing from birth and the enzyme defect lasts throughout life This is supposed to be an inborn error of metabolism although it has not been definitely proven At any rate it is extremely rare as is also the severe lactose intolerance with lactosuria These forms of lactose intolerance will therefore not have to be taken into account when the suitability of milk as a nutrient for large scale programme is discussed

(b) *Racial lactase deficiency* The first groups of humans used for studies on intestinal disaccharidases happened to be North Americans and North Europeans In these groups high intestinal lactase activity was found in general to persist throughout life When the studies were extended to other populations however it was discovered that in many races nearly all of the adults were lactose intolerant. This has e g been found true of Indians Greenland Eskimos Orientals etc (Table 2) In Africa it was revealed that Negroes of Bantu tribes were lactase deficient as adults while subjects of Hamitic origin retained their lactase activity throughout life This form of lactose intolerance thus seems to be bound to certain races or ethnic groups and it is usually named racial lactose intolerance

Adult subjects of those populations with racial lactase deficiency will not be fit to consume milk As infants however they possess intestinal lactase Up to a certain age it will thus be possible to feed these subjects milk *It is then important to know when their intestinal lactase disappears* Cook (13) has performed a careful investigation in Baganda infants this being one of the tribes showing racial lactase deficiency Lactose tolerance tests were performed at different ages The blood glucose response to lactose decreased gradually during the first years of life and in some exceptional cases lactose intolerance had already developed at six months of age The racial loss of intestinal lactase thus occurs rather early in childhood At the most can it be assumed that these subjects tolerate lactose during the first year of life The variation with age has however so far only been studied in this single population

It should be noted that this early loss of the tolerance for lactose occurred also in subjects which were well nourished and free from intestinal diseases As will be presented below malnutrition and diseases involving the small intestine can also influence the lactase activity

(c) *Secondary lactase deficiency* Inflammatory and degenerative diseases involving the

Table 2 *Frequency of adult lactase deficiency in some different population groups*

Group studied	n	with lactose intolerance	Authors
White			
USA	(16)	6	Dunphy Littman Hammond Forstner Dahlqvist & Crane (24)
	(50)	*16*	Sheehy & Anderson (37)
	(19)	*16*	Cuatrecasas Lockwood & Caldwell (17)
	(20)	5	Bayless & Rosenzweig (4)
	(100)	6	Newcomer & McGill (34)
Switzerland	*(18)*	*17*	Auricchio Rubino Landolt, Semenza & Prader (3)
England	(69)	20	McMichael Webb & Dawson (33)
Denmark	*(700)*	*5*	*Gudmand Höyer Dahlqvist & Jarnum* (27)
Sweden	(400)	< 1	Berg Dahlqvist Lindberg Meeuwisse & Nordén (5)
Finland	(248)	15	Jussila Isokoski & Launiala (31)
Negro			
USA	(41)	73	Cuatrecasas Lockwood & Caldwell (17)
	(20)	70	Bayless & Rosenzweig (4)
Africa			
Bantu	(52)	91	Cook & Kajubi (15)
Other tribes	(60)	9–44	Cook & Kajubi (15)
Greek Cypriots	(17)	88	McMichael Webb & Dawson (33)
Indians			
USA	(3)	67	Welsh Rohrer Knudsen & Paustian (39)
Oriental			
Australia	(20)	95	Davis & Bolin (23)
USA	(11)	100	Chung & McGill (11)
Greenland Eskimos			
Denmark	(32)	72	Gudmand-Höyer & Jarnum (28)

biopsy The mucosa is homogenized and analysed for disaccharidase activities (18) The activities are calculated as units (μmoles of substrate hydrolyzed per min at 37°C) either per g of protein (Table 1) or per g wet weight of the mucosa (Fig 2) The correlation between the disaccharidase activity found in a mucosal biopsy and the results of oral tolerance tests is very high

The normal range of mucosal disaccharidase activity

The range of disaccharidase activities in mucosal biopsy specimens from one control group of adult North Americans and one group with lactase deficiency is seen in Table 1

In most patients with lactase deficiency a low residual lactase of a few units per g protein is found (Table 1) Part of this residual activity is caused by a lysosomal β galactosidase which is probably not involved in the digestion of lactose (1, 2) In many patients, however, there is also a small residue of the brush border lactase (1) It is therefore important to define the limit of lactase activity below which the patient is intolerant to lactose In the investigation shown in Table 1 none of the lactose-intolerant subjects had higher lactase activity than 6 units per g protein while none of the control subjects had less than 9 units Other investigations have produced similar figures

The newborn infant seems to have the same proportions of the different disaccharidase activities as the adult controls shown in Table 1 In this respect man seems to differ from most animals, in which the lactase activity is high at birth, while the other disaccharidases are low or absent After weaning the animals lose their lactase and the other disaccharidases develop This development is *not* regulated by

spect Kerpel Fromus Jani & Fekete (32) have studied 62 Hungarian children with different degrees of malnutrition Only 4 of these were classed as kwashiorkor patients Oral tolerance tests showed that 75 of these malnourished children did not absorb lactose normally The frequency and the severity of the lactose mal absorption seemed to increase with the severity of the malnutrition Similar experience is re ported by Berkel Kiran & Say (6) These in vestigations seem to indicate that a milder de gree of malnutrition may also interfere with the intestinal digestion of lactose Cook (14) stated that in mild kwashiorkor mucosal damage and fall in disaccharidase activity are less com mon than in severe" kwashiorkor but never theless patients with mild kwashiorkor often develop permanent lactase deficiency too

Use of milk for treatment of protein malnutrition

The lactose intolerance in severe protein-calorie malnutrition plus the frequent occurrence of racial lactase deficiency in those parts of the world where protein malnutrition is commonly found seems to make the value of milk ad ministration in its present form somewhat doubtful Although patients with protein defi ciency will benefit from milk administration they will also experience intestinal symptoms provoked by its lactose content

How then can these difficulties be overcome? One apparent possibility is to replace milk by some other (vegetable) protein source of similar quality The carbohydrate source should then be monosaccharides sucrose maltose or starch. The intestinal sucrase and maltase activities are more powerful than the lactase and there is no racial deficiency in these enzymes It is therefore to be assumed that the patients with lactose intolerance due to various causes will be able to hydrolyze and absorb sucrose and maltose Nevertheless this should be tested in the patients before any large scale production of such nutrients is commenced

Another possibility is to remove the lactose from milk Some attempts have been made to remove lactose by dialysis but we do not re gard this as a suitable method as it entails the loss of almost half of the calories Further more there is a risk that other important com ponents of the milk may be removed as well Hydrolysis of the lactose into monosaccharides before the milk is fed should be a better pro position It is possible to add fungal lactase preparations to milk and preincubate the mix ture before it is fed (12 Dahlqvist & Meeu wisse—unpublished) This is however a too impractical and expensive method to be used on a large scale We have therefore tried to hydrolyze the lactose in milk by a continuous process utilizing insoluble lactase built into a perfused column (19) This appears to be a possible way although all practical details are not yet solved With this system the lactose in milk could be hydrolyzed during processing at the dairy in an equally simple way as Pasteuri zation is now performed This method would have the advantage that no nutrients are re moved from the milk and no additions (of fungal extracts) are made since the enzyme is not released from the column

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small intestinal mucosa often cause a secondary decrease of all the disaccharidase activities This is seen e g in tropical and non tropical sprue (21 26, 40) It has often been observed that, although this secondary decrease affects all the disaccharidase activities the lactase activity is usually proportionally more depressed than the others Since in normal lactose tolerant subjects the lactase activity is already weaker than the other disaccharidase activities (Table 1) it is natural that patients with secondary disaccharidase deficiency become primarily intolerant to lactose, and only in very pronounced cases do digestive disorders of other disaccharidases occur

After treatment of the primary disease, the disaccharidase activities usually return to normal values It seems however, as if in some cases the secondary lactase deficiency may become permanent Gray Walter & Colver (26) found that 63 % of the patients with apparently cured tropical sprue showed lactose intolerance, as opposed to 21 % in a control material

Moreover in severe protein calorie malnutrition (kwashiorkor) there is a secondary disaccharidase deficiency affecting the lactase activity more severely than the other disaccharidase activities (7, 16 25) The lactase deficiency in these patients has been demonstrated both by oral tolerance tests and by the assay of enzyme activities in mucosal biopsy preparations The change from milk to carbohydrate-free diet has been reported to produce a dramatic drop in stool weight in these patients (8 9) Monosaccharides and other disaccharides (sucrose and maltose) were well tolerated and it was therefore concluded that the lactase deficiency is a significant factor in causing the diarrhoea of kwashiorkor The lactose intolerance in kwashiorkor has been demonstrated by many other authors as well (29 30 35 38) (Table 3) Although reduced activity of the other disaccharidases and of monosaccharide transport can be demonstrated in patients with kwashiorkor (29, 30), the decrease in these activities will not result in sugar intolerance symptoms in most cases This is due to two factors 1) in the normal intestine there is much higher activity of the other disaccharidases (and presumably also of the glucose transport system) than of the lactase, and 2) the lactase activity for some unknown reason seems to be proportionally more depressed than the other activities in conditions causing secondary disaccharidase deficiency

It has to be pointed out that, in spite of the lactose intolerance of the kwashiorkor patients, they will benefit from milk administration (35) This has been observed by many different authors studying these problems However, the milk administration will give diarrhoea and abdominal discomfort, and furthermore it is very probable that the rapid passage of the milk through the intestine will result in suboptimal utilization of the protein as well as the other nutritional components of the milk

It should also be observed that the pathological changes in the small intestinal mucosa of kwashiorkor patients will persist for a very long time after the introduction of dietary treatment and the lactose deficiency may often become permanent Stanfield, Hutt & Tunnicliffe (38) found marked morphological alterations and low disaccharidase activities for upto 1 year after the onset of therapy, although clinical improvement had occurred within a few weeks Prinsloo Wittmann Pretorius Kruger & Fellingham (35) have reported similar observations Cook & Lee (16) studied patients cured of kwashiorkor 4–10 years before the time of the investigation In tribes which normally have high lactase activity throughout life the subjects cured of kwashiorkor had low lactase although the activities of the other disaccharidases were high (Fig 2) Apparently the subjects previously suffering from kwashiorkor had in this case a permanent lactase deficiency

There is thus no doubt that patients with severe kwashiorkor will be intolerant to dietary lactose and that this intolerance will frequently become permanent also in populations in which normally a high level of lactose is maintained throughout life We are less knowledgeable about milder forms of malnutrition in this re

spect Kerpel Fromus Jani & Fekete (32) have studied 62 Hungarian children with different degrees of malnutrition Only 4 of these were classed as kwashiorkor patients Oral tolerance tests showed that 75 of these malnourished children did not absorb lactose normally The frequency and the severity of the lactose mal absorption seemed to increase with the severity of the malnutrition Similar experience is re ported by Berkel Kiran & Say (6) These in vestigations seem to indicate that a milder de gree of malnutrition may also interfere with the intestinal digestion of lactose Cook (14) stated that in mild kwashiorkor mucosal damage and fall in disaccharidase activity are less com mon than in severe kwashiorkor but never theless patients with mild kwashiorkor often develop permanent lactase deficiency too

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In the high atresia group 7 patients died (25) and in the low atresia group there were also seven deaths (9 %) The combined mortality was thus 15

Other malformations were found in the high atresia group in 75 % of cases and in 40% of the low atresia group Seven of the 9 patients who died at operation had serious multiple defects

Among the patients with low atresia 17 cases of vestibular atresia were found and 39 cases of perineal fistula—21 of these being boys and 18 girls

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necessary in the 277 cases subjected to operation since myectomy has only been performed for two-and a half years

Even so the number amounting to 10% at most shows that in all operations in which sphincter myectomy is performed as a matter of routine this is done unnecessarily in 90% of the cases in which a simple sphincter dilatation would have been adequate

U Schafer (Bremen BRD) *The abdomino sacro-perineal approach in the treatment of the high supralevatory type of imperforate anus*

At the Surgical Clinic of the Children s Hospital Bremen 96 children with a supralevatory type of imperforate anus have been treated 76 cases were low supralevatory forms and 20 high supralevatory

In 76 of these 96 cases the abdomino-perineal approach was used and in 20 cases the abdomino-sacro-perineal approach As the results by the abdomino-perineal approach were not very satisfactory in the high supralevatory type we changed over to the abdomino-sacro-perineal approach in these cases We regard this as the best way to find the exact position of the pubo-rectal sling and consider that children with a high supralevatory type of imperforate anus will benefit from the additional approach Our intention is to restrict the combined procedure to high levatory types only and to treat the low or translevatory types by abdomino perineal pull through without using the sacral approach

MAIN TOPIC II

CARDIOVASCULAR SURGERY ON INFANTS

J Crafoord G S ttergren & S Soderlund (Stockholm Sweden) *Determination of oxygen saturation in the pulmonary artery during banding*

Constriction of the pulmonary artery known as banding is performed in patients with VSD with or without associated vitium cordis in whom cardiac insufficiency does not alter after treatment with digitalis and diuretics The aim is partly to reduce the left to right shunt so that the insufficiency is removed and partly if possible to prevent any increase in pulmonary vascular resistance

How tightly should the band be drawn? Various methods can be employed to arrive at an answer to this question It can be tightened until the heart stops and then released Alternatively the pressure in the pulmonary artery can be reduced to a certain level Or an attempt can be made to measure the flow with an electronic flow meter applied distally of the band

We have chosen a fourth method Our working hypothesis is that an endeavour should be made to achieve normokinetic pulmonary blood flow with basal metabolism In pre-operative catheterisation high oxygen saturation in the pulmonary artery indicates hyperkinetic pulmonary circulation Thus it seems best to tighten the band at operation until a normal value for oxygen saturation is reached We have done this in a number of cases However the apparatus employed for measuring the oxygen saturation could have been more accurate The patients were anaesthetised with halothane-oxygen-nitrous oxide Blood samples were taken with a fine needle the mean pressure being registered at the same time After the operation the patients reacted favourably and the cardiac insufficiency was found to have disappeared Our results indicate that it is difficult if not impossible to predict the pressure range at which normokinetic perfusion will be obtained

L E Carlgren & S Hagberg (Goteborg Sweden) *Cardiovascular infant surgery Indications methods and results*

At the Surgical Clinic of the Children s Hospital Goteborg 73 infants under 1 year of age hav been operated upon for cardiovascular

Thirty-eight of the patients were male, and 22 female Fifty five cases could be classified as anal or rectal agenesis 27 being of the latter type

The results of treatment were excellent in 13, good in 10, satisfactory in 7, and poor in 6 of the surviving patients No follow up examination was made in respect of seven patients, and there were 17 deaths

In 21 cases additional congenital anomalies complicated the situation In 17, the other mal formations were potentially lethal and 13 of these cases terminated fatally In 4 patients death was attributable to technical errors or failure to assess the condition adequately

In 10 of the patients with imperforate anus the presence of Hirschsprung s disease was later confirmed or strongly suspected

L Lindell & T Aalto (Turku Finland) *Defaecography Cine fluorographic study of patients with Hirschsprung s disease after operation*

A cine film was presented showing by fluorographic means the emptying of the rectum in one case operated on by the Soave technique and six by the Duhamel procedure Some aspects of normal bowel and sphincter function were pointed out to aid recognition of the hypo ganglionic segment scar contracture of the sphincter and relaxation of the pelvic dia phragm in the cases concerned Mention was also made of some difficulties and risks in volved in the method

H C Sommerchild & A Bjorkheim (Oslo Norway) *Intestinal perforations as a complication in exchange transfusions in neonates*

Exchange transfusion through the umbilical vein is nowadays the standard treatment of neo natal hyperbilirubinaemia However, the risk of intestinal perforation as a complication in this procedure is a new observation mentioned for the first time by Herman in 1965, but re ported in a further 13 cases during November 1968 Most of the perforations have occurred in the colon

The author presented another case, with a review of 20 cases from the literature There was a survival rate of 75%, in contrast to 15% survival from spontaneous colonic per forations without previous exchange transfu sion This suggests that as regards pathogenesis the exchange transfusion cases differ from cases of spontaneous enteric perforation

It was suggested that mechanical factors con stitute the most important common denomi nator in cases following the exchange proce dure Stress was laid upon some observations *which support this suggestion*

T Norman & B Otnes (Oslo Norway) *Diffuse intestinal ganglioneuromatosis*

Two cases of diffuse intestinal ganglioneuroma tosis are reported In one patient, a 15 year old boy, the lesion was associated with medul lary carcinoma of the thyroid severe diarrhoea and mucosal neuromas Three previous reports have been published of patients with a similar syndrome In all of them additional bilateral phaeochromocytomas were found

The second patient a 3 year old girl ex hibited not only diffuse ganglioneuromatosis but also diffuse intestinal lymphoid polyposis but with none of the other stigmata of the syndrome mentioned above

U Schafer (Bremen BRD) *Sphincter myectomy in Hirschsprung s disease*

Since 1951 315 cases of Hirschsprung s dis ease have been treated at the Children s Hospi tal Bremen In 277 cases intraabdominal ante rior resection was performed In 11 of these cases (4 5%) sphincter myectomy was per formed as even three sphincter dilatations after the resection did not lead to regular evacuation of the bowel The results after sphincter myec tomy were satisfactory in 10 cases the stub born constipation was overcome *completely*

In the light of experience it is possible that an equal number of myectomies will still be

risation and angiocardiography showed partial anomalous venous drainage from right lung to right atrium and an obliterated left main pulmonary artery but no septal defect At the age of 7 years a second operation was performed during cardio-pulmonary by pass The pulmonary veins were transposed to the left atrium At follow up after 1 year the child s condition was quite satisfactory The combination of anomalies seen in this case has to our knowledge not previously been reported

2 Cyanosis at birth Systolic and diastolic murmurs At 3 weeks right middle lobe (RML) emphysema and clinical cardiac examination led to a diagnosis of pulmonary stenosis and ventricular septal defect At 10 weeks bronchography performed on account of respiratory distress increasing cyanosis and cyanotic spells showed a stenosed RML bronchus and cardiac catheterisation confirmed the clinical diagnosis The stenosed bronchus was shown to be displaced by a prostenotic dilated pulmonary artery A RM lobectomy was performed as an emergency operation Follow up at 6 months of age was satisfactory

3 Tachypnoea and cyanotic spells since birth LUL emphysema Lobectomy at 3 weeks of age on vital indications Postoperatively RML emphysema and RUL atelectasis RML bronchus possibly stenosed by aberrant artery Bilobar emphysema has been established in this case

It is of importance to diagnose the association of infantile lobar emphysema and a cardiovascular anomaly In some cases the treatment of the cardiovascular condition may have a marked influence on the development of lobar emphysema and hence on the prognosis of the patient The diagnostic armamentarium should include bronchography cardiac catheterisation and angiocardiography

G Semb & K V Hall (Oslo Norway) *Waterston shunts*

Shunts from the systemic artery to the pulmonary artery with the technique described by Waterston has great advantages especially in paediatric surgery where this is indicated The operation was performed on 10 patients under 2 years of age 5 cases of Fallots tetralogy 3 cases of pulmonary atresia and 2 cases of tricuspid atresia The indications for operation were increasing cyanosis cyanotic spells pulmonary infections or progressive cardiac failure

The operative method was described in detail with particular reference to the size of the shunt

The clinical results were discussed and related to the objective improvement in hypoxia and polyglobulinaemia

The postoperative complications and mortality and morbidity figures were presented

FREE PAPERS

K V Parkkulainen (Helsinki Finland) *Ciné urethroscopy in boys*

A film was presented with the aim of demonstrating the modern possibilities of endoscopic filming in children and even in infants Hitherto the interpretation of what is seen at endoscopy has been entirely dependent upon the subjective understanding and personal experience of the urologist who has had no opportunity for a subsequent detailed study of the appearances Endoscopic filming allows examination of the wide variations observable in normal urethras and comparison of the findings with those in voiding cystography At the same time documentation of normal and pathological conditions has become possible Six cases were presented together with the respective voiding films

R. Stenstrom & J Elo (Helsinki Finland) *Nephrobarinosis after micturition urethrocystography (UCGI) in children*

Among 1 600 UCG examinations of children made at the Aurora Hospital during the period 1957–1969 3 cases of nephrobarinosis were found after urethrocystography with a sterile barium suspension Nephrobarinosis must be

malformations since 1965 Of these, 25 have died

Twenty cases of patent ductus arteriosus have been operated on, with only one death, which resulted from aspiration This low mortality must be set in relation to the fact that the indications for operation were wider in those patients who did not exhibit any signs of concomitant cardiac malformation than in those who did In 22 cases of pulmonary hyperfusion due to other left to right shunts, pulmonary banding was carried out, with 11 deaths In these cases the lesions were extremely severe and without surgery the mortality would probably have approached 100 per cent

Ten patients were operated upon for coarctation of the aorta, and 3 of these died Of the survivors, 3 still have a high pressure gradient between the arms and legs and will probably have to undergo re operation in the future The remaining 4 patients are alive and in good condition Seven cases of pulmonary atresia or severe stenosis with intact ventricular septum were treated, with four operative deaths We have applied both open and closed methods, with a dilator

Five patients with transposition of the large vessels were operated upon by the Blalock-Hanlon method One of these died immediately after the operation, and another during a later operation The remaining 3 patients are alive and in fairly good condition Finally a shunt operation was performed in 9 cases with cyanotic heart disease and pulmonary hypofusion with four deaths

O Vilkki (Turku Finland) *Cardiac surgery in infants at Turku University Hospital*

Since 1962 48 patients under 2 years of age have been referred to the pediatric surgical services of Turku University Hospital for refractory cardiac failure In every case, the condition of the patient was deteriorating despite adequate medical treatment and the prognosis was considered extremely poor In many of the cases, life could be maintained only by intensive care and/or respiration treatment before surgery Of the total series of 48, 23 were restored to normal health by corrective operation Seventeen were definitely improved by palliative operation In 8 patients surgery did not alleviate the cardiac distress and 6 of them died A patent ductus arteriosus was the main reason for the cardiac failure in 17 patients On follow up examination the cardiac status was virtually normal in all of them

Coarctation of the aorta was the reason for the heart failure in 9 patients Five were cured, three recoarctations were observed after initial improvement and 1 patient died

Pulmonary hyperaemia, with VSD as the underlying lesion was relieved by pulmonary banding in 10 patients One case complicated by coarctation proved fatal All the others showed noteworthy postoperative improvement Electro-encephalographic monitoring was used as an index of adequate brain oxygenation to determine the tightness of the band

Pulmonary hyperaemia due to Fallot s pentalogy was palliated by aorto-pulmonary shunt in one of them and by right subclavio pulmonary shunt in 3 patients All of them improved

Other anomalies consisted of truncus arteriosus communis (3) aorto-pulmonary window (1), tricuspid atresia (1) ectopia cordis (1) and vascular ring (1) Of these only the last was cured by surgery and 4 patients died

G Semb T Kluge, S Sørland & S Ek (Oslo, Norway) *Infantile lobar emphysema Three cases with associated congenital cardiovascular anomalies*

Infantile lobar emphysema is a dangerous disease and therefore of importance The clinical picture and radiographic appearance are characteristic The three cases with associated congenital cardiovascular anomalies were

1 LUL emphysema LLL atelectasis rotation dextrocardia and right sided aortic arches At the age of 3 years LU lobectomy was performed Pulmonary infections continued and a systolic murmur was detected Cardiac cathete-

risation and angiocardiography showed partial anomalous venous drainage from right lung to right atrium and an obliterated left main pulmonary artery but no septal defect At the age of 7 years a second operation was performed during cardio-pulmonary by pass The pulmonary veins were transposed to the left atrium. At follow up after 1 year the child's condition was quite satisfactory The combination of anomalies seen in this case has to our knowledge not previously been reported

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regarded as a relatively grave complication, for it can lead to permanent damage to the kidney Persistent proteinuria and haematuria may result In one of our cases acquired hypoplasia of the corresponding kidney was found 1 year after the examination in this case massive infection was apparent just after the UCG The authors recommend that the use of suspensions of barium sulphate for UCG be abandoned

E Edelman & P Vilkki (Turku Finland)
Pressure measurement in the urinary tract

In the Department of Paediatric Surgery of the University Hospital, Turku, pressure measurements have been made in the distal ureters and bladders of children with radiologically demonstrated vesico ureteric reflux, and in non refluxing ureters and bladders of children examined for other urological reasons The children were all free from urinary infection at the time of examination The pressure measurements were made during normal cystoscopy by application of the same standard method Indwelling catheters with an external diameter of 1 mm have been employed and the pressures recorded with an Electromanometer EM 31 manufactured by Elema-Schonander The pressures in the distal ureter and in the bladder were recorded simultaneously with the degree of filling of the bladder with saline solution

The series examined consisted of 23 ureters With the bladder empty the functional efficiency of the ureter which is reflected in pressure alterations, seems to be inferior in the refluxing ureters to that of the non-refluxing ureters The functional efficiency in the refluxing ureters also seems to be impaired while the bladder is being filled although this is not normally the case in non refluxing ureters

T Parvinen, O Nielsen & R Nielsen (Turku, Finland) *Ectopia vesicae and duplication of the bladder in the same patients*

A presentation was made of 2 cases of duplication of the urinary bladder combined with exstrophy of the ventral compartment, along with a short anatomical and embryological survey

O Lindfors & R Anttila (Helsinki, Finland)
Experience with seven renal homotransplantations in children

Although kidney transplantation is the accepted and established therapy in the routine treatment of renal failure the literature on paediatric kidney transplantations is sparse The inevitable immunosuppressive treatment may be expected to retard the normal development of the child The prognosis is thought to be poor Since 1967, the kidney transplantation team at the University of Helsinki has performed kidney homotransplantations on seven children of ages ranging from 9 to 15 years All the patients had severe uraemia, including hypertension and retinal changes All the donors were parents Immunosuppression was maintained with prednisone 0 1–0 3 mg/kg/day and azathioprine 1 0–2 0 mg/kg/day The follow up has now covered a period of 14–42 months (in 4 patients, more than 2 years) One month after the transplantation 1 patient died of gastro intestinal haemorrhages and peritonitis His kidney function however was quite normal Four patients have had only one rejection, usually 3–6 weeks after transplantation Thus only 2 children have had more than one rejection In instances of rejection the therapy applied comprised not only higher doses of prednisone and azathioprine but also local irradiation of the graft (total dose 600–1 000 R) and Actinomycin C (200 μg) every other day until the rejection was halted Other complications observed were the nephrotic syndrome in one case urinary complications in 2 patients and encephalitis in one Four patients now have normal blood pressure and the other two moderate hypertension Serum creatinine is normal and retinal changes have almost regressed Slight osteoporosis has been observed The skeletal age is somewhat lower than normal but in 1 patient is 2 years in advance of the chrono-

logical age Growth in height has been minimal The gross appearance is more or less Cushingoid except in one child who had better compatibility and has needed less suppression therapy The surviving patients (6/7) all attend school move actively and are no longer bound to dialysis centres Most of the routine checks are made at local hospitals It is too early to draw conclusions about the future of children who have undergone kidney transplantation but the major problem seems to arise from the side effects of immuno-suppression In future even more attention should be paid to optimal immunological matching

B H Thomasson & M M Ravitch (Illinois USA) *Foetal surgery in the rabbit*

The film shown demonstrated the technique of endotracheal intubation laparotomy and hysterotomy in a pregnant doe Further foetal ureteral ligation and small bowel transection on the 25th day of gestation were shown the former was carried out entirely in utero and the latter by temporary extrusion of the foetus in its intact membranes through the hysterotomy

The technique was developed during the course of about 400 operations on foetuses with the aim of elucidating the foetal response to trauma and the pathogenesis of polycystic kidney and meconium peritonitis The foetal survival rate was about 60%

J Viljanto & S M Viitanen (Turku Finland) *Thermographic observations of wound healing in children*

The skin temperature around an inguinal or abdominal wound was studied by means of thermovision in 24 children under 13 years of age and in 5 adults aged 18-67 years Most of them had undergone operation for inguinal hernia or for incomplete descent of the testis Two patients with pyloric stenosis and two with Hirschsprung s disease were included The wounds were covered with Nobecutan® film which being in direct contact with the skin did not prevent its thermal radiation

It was observed that in the group of the infants under 1 year of age the wound was visible as a cool line for about a week The surrounding skin was warmer for 3-5 days postoperatively In the group aged 2-4 years the cool wound line was visible for 10-15 days and the warm area around it for about 3 weeks However the monophasic thermal curve of the younger patients was now replaced by one of biphasic form which attained the first maximum on the 5th day and the second on the 12th-14th day In children aged 8-12 years the biphasic nature of the increasing skin temperature around the wound was even more pronounced The peak values were attained during the first 10 days and again during the 3rd or 4th week The wound was seen as a cool line for 3 weeks

The first phase of increased skin temperature around the wound might be attributable to vasodilatation during the period of so-called post traumatic inflammation The second phase could be regarded as an expression of the high metabolic activity of connective tissue cells although many other causes might be suggested

R Bjordal (Oslo Norway) *Septic candida albicans infection*

During the period January 1969 to February 1970 septic *Candida albicans* infection was observed in 15 children whose ages ranged from 2 weeks to 12 years in the Department of Paediatrics at Rikshospitalet Eleven of these children had been admitted to the section for paediatric surgery

Thirteen of the patients succumbed and in these the septic Candida infection was disclosed at autopsy In 2 children, the diagnosis was made intra vitam Fungizone treatment was begun and the patients survived

Common denominators in these cases were bacterial infection deterioration treatment with antibiotics parenteral alimentation

E S Heikkinen & M Sulamaa (Helsinki, Finland) *Habitual dislocation of the hip*

A report was presented on two children, a boy and a girl, with habitual dislocation of the hip They were examined and treated at the Children s Hospital, University of Helsinki In the boy, both hips were affected, and in the girl, the right one

The main symptom was snapping or popping noises of the hip, which in both cases started at the age of 1 5 years In infancy, the hips were clinically normal The children had no trauma, no dysplasia in the hip joints, no capsular defect, and no neurogenic disorders The boy and the girl could each dislocate and reduce the hip at will The provocation test was positive at the age of 5 years in both patients At that time, the posterior dislocation of the femoral head could be seen, felt heard, and confirmed by X ray examination Initially the boy was treated unsuccessfully with an abduction splint After 6 months this treatment was discontinued, but the boy recovered spontaneously at the age of 8 years He is now 16 years old, and completely free from symptoms arising from the snapping hip, the girl had muscular atrophy in her right thigh, with occasional pain, and limped slightly In view of the progression of these symptoms, it was decided to operate Now 1 year after the operation the joint is stable, and its movements are normal

S Einola, P Vilkki, E Melartin & B Thomasson (Turku, Finland) *Luxatio congenita patellae*

A film was shown to present a case of congenital lateral dislocation of the patella in an 11-year old boy with progressively worsening gait and flexion contracture of the knee The correction of this condition by means of retinacular release and ligamentoplasty was demonstrated The report included a follow up examination, 8 to 15 months after surgery of 5 similar patients, 3 to 16 years old

G R Wallgren
Aurora Hospital
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Finland

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BOOK REVIEWS

G Debrun & C Gasquet *Explorations vasculaires des tumeurs abdominales de l'enfant* L'Expansion Paris 19 0 92 pp illus F 40

This book belones to a series of monographs published by the journal Annales d Radiologie Its first part (written by Debrun) reports the experience with abdominal angiography in 26 cases of benign tumors and 77 cases of malignant neoplasms most of which originated in the retroperitoneal ganglia the kidney the adrenal or the liver The findings are exemplified by instructive reproductions The second part (by Gasquet) is concerned with lymphography of 105 cases of malignant disease and is equally well illustrated The majority of these tumors were neuroblastomas or renal or ovarian neoplasms.

Though lymphography and abdominal angiography are now widely used in the exploration of malignant disease in adults experience with these diagnostic methods in infancy and childhood is still limited The book is therefore a useful contribution to the literature in an important and expanding field of pediatric radiology

G Theander

Nancie R Finnie *Børn med cerebral parese* Borgens Forlag Copenhagen 1970 192 pp illus D Kr 36 00

Nancie R Finnie is a physiotherapeut working at the Bobath Institute She has vast experience in the treatment of cerebral palsied children according to the Bobath principles The book is intended to be a guidance for both parents physiotherapeuts and other therapeuts

This book supplies a long felt want and it is very aluable that the book is now translated into Danish by Karen Bysted a physiotherapeut with many years experience in the treatment of CP children The translation is well adapted to Scandinavian circumstances and made with great understanding of the author s intentions

The book is illustrated with instructive drawings which make it easy to read and thus avoiding too difficult explanations and medical terms There are separate chapters about bathing feeding and other daily activities and also suggestions about different technical aids

The importance of cooperation between parents and therapeuts is emphasized To avoid confusion the parents must have the opportunity to discuss appropriate sections of the book with therapeuts This is a valuable book giving a better understanding of CP children s problems and good advices for their treatment

Ing id Bjerre

W Ludwig *Das Rechts Links Problem in Tierreich und beim Menschen* Mit einem Anhang "Rechts Links-Merkmale der Pflanzen" Springer Verlag Berlin, Heidelberg and New York 1970 496 pp illus.

This work was originally published as volume 27 of Monographien aus dem Gesamtgebiet der Physiologie der Pflanzen und der Tiere 1932 and the present edition is an unamended reprint. The work is thus almost 40 years old but nevertheless it gives an excellent summary of the problems concerned with the asymmetric development of a great number of animals including man The work is divided into three parts (1) An introduction of 29 pages being mainly a matter of terminological contents (2) A thorough account of the asymmetry within the animal kingdom and particularly in relation to man (3) A general discussion on the problems Some of the illustrations of asymmetrical development in the lower invertebrates appear somewhat problematic but they have significance in general and do not detract from the value of the work The greatest value for physicians will probably be found in the paragraphs concerning left handedness and other consequences from inverse asymmetric development in man Unfortunately the subject on psychological aspects (inferiority complexes etc) has only been briefly touched on

G Mandahl Barth

R Burkhardt *Farbatlas der klinischen Histopathologie von Knochenmark und Knochen* Springer Verlag Berlin Heidelberg and New York 1970 115 pp illus DM 248 —

This beautiful and expensive atlas of bone marrow histopathology is based upon the experience gained from 2 200 biopsies Although the size of the author s material does not seem impressive the high quality of the sections produced in his laboratory is obvious from the 700 microphotographs in colour which form the bulk of the volume An introductory chapter gives a detailed description of biopsy technique and the method used for cutting and staining the undecalcified bone marrow sections

Part of the atlas clearly suffers from superfluousness of illustrations Several microphotographs are thus devoted to vascular changes which certainly look the same in the bone marrow as elsewhere in the body For instance a cross section of a muscular artery whether dilated or contracted is hardly warranted in an atlas of bone marrow histopatho ogy Three microphotographs serve to illustrate the well known appearance of vascular amyloidosis One whole

page—ten microphotographs—claims to show various phases of thrombus formation

The various aspects of genuine bone marrow pathology i e disturbances of the hematopoietic and reticuloendothelial systems which naturally occupy the greater part of the atlas are outlined with considerably more care and accuracy However the general question might be raised whether this type of textbook (or atlas) should be written by a pathologist with a particular interest of hematology rather than by a clinical hematologist interested in histopathology It is the reviewers biased opinion that bone marrow histopathology like dermatohistopathology and any other special field of pathology is best managed by individuals with a broad experience of general pathology

Bengt Robertson

F C Fraser & V A McKusick (eds) *Congenital malformations* Proceedings of the Third International Conference The Hague September 1969 Excerpta Medica Amsterdam 1970 466 pp illus US$ 27 50

The science of congenital malformations teratology has been the subject of three international congresses—the first was held in 1960 the second in 1963 and the third in 1969 The reports of all three congresses have been published in the Excerpta Medica International Congress Series and give admirable summaries of the development of interest in and knowledge of this subject The third volume contains a number of very good review lectures most however of a fairly superficial nature Some basic fields related to teratology have been summed up under the heading of Developmental Biology 1969 Little really new data are given but the summaries presented may be of value for the non specialist to keep track of what happens in these rapidly expanding fields Other reviews deal with the uterine milieu and the early embryo and the remaining ones are dedicated to human teratology Besides such review articles the chairmen of a number of parallel discussion and paper sessions have given short summaries of the original data presented or the discussions held I found these chapters to be of limited value becaus of their superficiality

This volume is good reading for anyone who wants to make himself acquainted with the present state of the international research dealing with malformations It should be of interest not only to the embryologist and teratologist but also and perhaps still more to the pediatrician and other specialists who want to be informed on basic research in this important field

Bengt Kallen

O Gross e & R. Ortmann *Grundriss der Entwicklungsgeschichte des Menschen* 7th Ed Springer Verlag Berlin Heidelberg and New York 1970 207 pp illus DM 28 —

Knowledge about human embryology is an important basis for the understanding of both adult anatomy and the origin of anatomical deviations and malformations Human embryology is therefore an essential part of the medical curriculum There are many textbooks available varying in both size and quality The 7th revised edition of a classical German text book has now been published It describes the formal genesis of the human body from fertilization to birth and is well illustrated with numerous microphotographs diagrams and reconstructions The descriptions are relatively brief and give the bare basic knowledge for the student—in some sections e g the clinically important section of urogenital tract development The text is not quite up to-date

The author has tried to introduce some more modern aspects on development by brief references to cytogenetics experimental embryology teratology etc These discussions are very short As the book is especially suitable for medical students more information on the common malformations and their embryological origin would have been welcome if only to increase the motivation for the students to study embryology On the other hand the final chapter on Geburtszustand" with a summary of the Portmann ideas could from this aspect well be omitted

Bengt Kallen

ANNOUNCEMENT

The 3rd European Congress of Pediatric Neurosurgery takes place in Gottingen West Germany from 21st to 23rd September 1972 For information please address Prof Dr med K A Bushe Direktor d Neurochirurgischen Klinik der Universitat Gottinge 3400 Gottingen (West Germany) Gosslerstraße 10

Acta Pædiat Scand 60 505–511 1971

EFFECTS OF DIET ON FATTY ACID COMPOSITION OF PLASMA AND RED CELL PHOSPHOGLYCERIDES IN THREE MONTH OLD INFANTS

RAGNAR OLEGARD and LARS SVENNERHOLM

From the Department of Paediatrics and the Department of Neurochemistry Psychiatric Research Centre University of Goteborg Goteborg Sweden

It has been shown by Arild Hansen and his school (6 8) that insufficient dietary linoleic acid will result in clinically manifest essential fatty acid deficiency in the human infant. Hansen et al (8) also found that the blood serum levels of polyunsaturated fatty acids reflected the dietary intake of linoleate remarkably wel From parall l animal experiments headed by Ralph Holman methods were developed which were applied in the estimation of the linoleate requirements of human infants (10) The estimated minimum requirement of linoleate was approximately 1 4 of total calories

Infants fed cow s milk formulas consisting of cow s milk diluted 1 1 with water and containing added sucrose may show biochemical signs of insufficient dietary linoleate (11 16 18) But during the last decade industrially manufactured cow s milk formulas used instead of breast milk in Scandinavia and several other Western countries contain supplementary linoleate In Sweden the linoleate concentration used is approximately the same as that in breast milk or at least 4 calorie Only relatively few studies are available on the effect of linoleate fortified formulas on the blood fatty acid pattern Compared with the level in breast fed infants Hansen et al (7) found that a cow s milk formula with 7 2 calorie linoleate enhanced the plasma lipid linoleic a id but suppressed arachidonic acid while Mendy et al (16) reported that a formula with 5 5 calorie dietary linoleate raised both of the two acids in plasma lipids The observation periods were short in these studies particularly in the latter The controversial results of the two studies and the lack of knowledge of the effect of the long term use of a linoleate-enriched formula prompted us to undertake this study The dietary period selected was the first 3 months of life i e a period long enough to reduce the effect of the composition of the fat depots at birth and short enough to preclude any effect of dietary supplements to the milk diet

MATERIAL AND METHODS

Clinical material

Formula fed infants Nine apparently healthy and normally developed infants in an infant nursery (Myrornas spadbarnshem Goteborg) were arbitrarily selected for the study The infants were fed formulas based on skimmed cow s milk fortified with vegetable fat (Findus Tillagg Findus AB) The composition of the formula is given in Table I Fruit juices had been added to the diet from 8 weeks of age One infant was found to have hypercholesterolemia and an abnormal cholesterol phospholipid ratio and was therefore excluded This left eight infants for the present study

Breast fed infants One healthy infant in the same infant nursery as above was given pooled breast milk from the Breast Milk Central Goteborg. Nine healthy infants were arbitrarily selected in a Welfare Centre for infants and children in Goteborg These infants received breast milk from their own mothers and an addition of fruit juices from 6 to 8 weeks of age

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The 3rd *European Congress* of Pediatric Neurosurgery takes place in Gottingen West Germany from 21st to 23rd September 1972 For information please address Prof Dr med K A Bushe Direktor der Neurochirurgischen Klinik der Universitat Gottingen 3400 Gottingen (West Germany) Gosslerstraße 10

Table 3 *Mean concentration of plasma lipids of newborn infants 3 month old infants and adults mg/100 ml*

	Newborn infants (17) (n ?0)		3-month-old infants				Normal adults (22) (16–38 years)			
			Breast fed (n = 10)		Formula fed (n = 8)		Women (n = 28)		Men (n = 62)	
	Mean	S D	Mean	S D	Mean	S D	Mean	S D	Mean	S D
Total lipids	257[a]	59	609[a]	91	637[a]	82	648[b]	114	610[b]	120
Triglycerides	39	?0	168	72	211	64	88		84	34
Cholesterol	66	18	153	29	147	17	185	38	192	57
Phospholipids	113	24	.01	38	194	25	232	44	208	34
Cholesterol/phospholipid ratio	0 59	0 07	0 77	0 08	0 76	0 07	0 80		0 95	

Calculated [b] Gravimetric determination

fants was still larger in red cell lecithin than in plasma phosphoglycerides

The concentration of the fatty acids of the linolenic acid series in the formula fed infants was half of that in the breast fed infants in whom it was in the range for normal Swedish adults (2 17) Among the individual fatty acids of the linolenic acid series the difference was largest for the most poly unsaturated acid of the series 22 6 $(n-3)$

Fatty acid composition of red blood cell cephalins

The concentration of polyenoic acids was significantly higher in the formula fed infants (Table 5) The sum of fatty acids of the lino-

Table 4 *Fatty acid composition of plasma phosphoglycerides and of red blood cell lecithin in breast fed and formula fed 3 month old infants*

	Plasma phosphoglycerides						Red blood cell lecithin					
	Breast fed (n = 10)		Formula fed (n = 8)		Difference Formula fed Breast fed		Breast fed (n = 7)		Formula fed (n = 8)		Difference Formula fed Breast fed	
Fatty acid	Mean	S D	Mean	S D	Mean		Mean	S D	Mean	S D	Mean	
16 0	28 5[a]	3 84	24 5	2 09	−4 0[b]		34 6	1 11	31 0	4 41	−3 6	[b]
16 1	1 9	0 82	1 50	0 35	−0 4	—	2 2	0 54	1 7	0 32	−0 5	
18 0	18 2	0 72	14 0	2 27	4 ⌄		14 8	0 78	11 3	1.55	−3 5	
18 1	14 0	0 62	16 8	3 21	+2 8		21 6	1 10	21 8	1 28	+0 2	—
18 2 $(n-6)$	20 0	1 13	27 3	0 96	+7 3	• •	13 2	1 46	22 2	1 90	+9 0	
18 3 $(n-3)$ + 20 1	0 7	0 13	0 7	0 16	±0	—	1 0	0 24	0 8	0 12	−0 2	
20 3 $(n-9)$	traces		traces				traces		traces			
20 3 $(n-6)$	2 5	0 43	3 5	0 39	+1 0	•	1 8	0 31	2 2	0 28	+0 4	••
20 4 $(n-6)$	8 7	1 64	7 7	1 34	−1 0	—	7 4	1 57	6 2	1 23	−1 2	—
20 5 $(n-3)$	0 5	0 18	0 3	0.26	−0 2		0 3	0 08	traces		−0 3	
2 4 $(n-6)$	0 3	0 11	0 9	0 14	+0 6		0 5	0 18	0 8	0 27	+0 3	•
22 5 $(n-6)$	0 2	0 05	0 9	0 18	+0 7		0 1	0 04	0 5	0 17	+0 4	
22 5 $(n-3)$	0 7	0 29	0 3	0 17	−0 4		0 4	0 06	0 2	0 09	−0 2	
22 6 $(n-3)$	3 8	1 36	1 4	0 26	−2.4		2 1	0 6?	1 0	0 36	−1 1	••
18–22 $(n-6)$	31 7	2 09	40 4	1 32	+8 7		22.9	1.38	32 1	1 94	+9 2	
18–22 $(n-3)$	5 6	1 13	2 6	0 17	−3 0		3 9	0 67	2.1	0 48	−1 8	••
$(n-6)+(n-3)$	37 2	3 16	43 0	1 63	+5 8	•	26 8	1 75	34 1	0 97	+7 3	•

[a] Values are weight percentages of methyl esters
[b] S gnificance levels —0 05 level —0 01 level —0 001 level

Table 1 *Composition of breast milk and of the cow's milk formula*

	Breast milk	Formula
Calories/l	690[a]	670[b]
Percentage of calories		
Protein	7[a]	14[b]
Carbohydrates	42[a]	53[b]
Fat	51[a]	33[b]
Saturated fatty acids	21[c]	11[c]
Monoenoic fatty acids	20[c]	12[c]
Linoleic acid	3 5[c]	5 4[c]
Linolenic acid	1 2[c]	0 3[c]
Arachidonic acid	0 5[c]	0 2[c]

[a] Data from I G Macy & H J Kelly (15)
[b] Analysed by Findus AB Laboratories Bjuv Sweden
[c] Own GLC analyses

The concentrations of linoleic linolenic and arachidonic acids from a pooled sample of breast milk are given in Table 1

Age and weight on the day of blood sampling birth weight and gestation age are given in Table 2 The pregnancies and deliveries of the mothers had been uneventful Neonatal life had also been uncomplicated No infant had any known illness at the time of blood sampling

Blood sampling

Blood samples obtained from the internal jugular vein or as freely flowing blood from an incision in the sole of the foot were collected directly in heparinized test tubes Plasma was separated from blood cells generally within 4 hours and afterwards treated in the way described previously (17)

Chemical methods

The preparation of lipid extract quantitative assay for lipid P cholesterol and triglycerides separation of lipids by column chromatography with silicic acid methanolysis of phosphoglycerides and GLC of methyl esters were performed exactly as described in a previous paper (17)

Statistical methods

The significances of the differences in fatty acid concentrations and concentrations of main lipid classes between the two groups were tested by direct *t* test of the means

RESULTS

Concentration of main lipid classes in plasma

The mean values and standard deviations found for the two groups of infants are given in Table 3 The total lipids were calculated as the sum of the various lipid classes The cholesterol figure was multiplied by 1 44 to include the fatty acids in cholesterol esters For free fatty acids, a figure of 20 mg/100 ml was added

No significant difference in the concentration of any lipid class was found between the breast fed and the formula fed infants The mean triglyceride concentration was somewhat higher in formula fed infants

Cholesterol and phospolipid levels had almost reached normal adult levels but the triglyceride level was about twice as high as the adult level

The cholesterol/phospholipid ratio was close to that for adult women (Table 3)

Fatty acid composition of plasma phosphoglycerides and red blood cell lecithin

The plasma concentration of polyenoic acids was significantly higher in the formula fed group while the concentration of saturated fatty acids was higher in the other group (Table 4) The concentration of linoleic acid and the sum of all fatty acids of the linoleic acid series was about 30% lower in the breast fed infants But there was no significant difference in the concentration of arachidonic acid between the two groups

The red cell lecithin content of polyenoic acid was also significantly higher in the formula fed infants The difference in the concentrations of fatty acids of the linoleic acid series between the breast fed and the formula fed in

Table 2 *Age and weight at blood sampling birth weight and gestational age*

	Mean	Range
Breast fed infants (*n* = 10)		
Age	96 days	82–116 days
Weight	5 670 g	4 650–6 600 g
Birth weight	3 620 g	2 750–4 180 g
Gestational age	40 0 weeks	38–43 weeks
Formula fed infants (*n* = 8)		
Age	100 days	88–117 days
Weight	5 280 g	4 500–5 880 g
Birthweight	3 440 g	2 750–4 180 g
Gestational age	40 0 weeks	38–41 weeks

hydrate/fat ratio in the formula than in the breast milk (1) At birth the triglyceride plasma concentration is very low and at one year of age the triglyceride level[1] is the same as in adulthood (Table 3) The 100% higher plasma triglyceride concentration of our 3 month old infants coincided in time with the very high glucose tolerance found by v Euler et al (3) in this age group These authors suggested "a relative hyperinsulinism" at this age which could also explain the hypertriglyceridemia in the infants since a high correlation has been demonstrated between the insulin response to high carbohydrate diet and the degree of hyper triglyceridemia (20) in adults

Fatty acid composition

The fatty acid composition of plasma phosphoglycerides and red blood cell lecithin of the 3 month old infants had changed from the new born to the adult pattern in both groups of in fants Linoleic acid (18 2 (n−6)) had in creased and arachidonic acid (20 4 (n−6)) had diminished The two groups did not differ significantly from one another in concentration of arachidonic acid in lecithin which was within the range for the adult Swedish popula tion (2 17)

There were two major differences in the fatty acid composition of plasma and red cell lecithin between breast fed and formula fed in fants (Table 4) The sum of the polyenoic fatty acids was smaller in breast than in formula-fed infants and the ratio of the fatty acids of the linolenic acid to the linoleic acid series was lower in the formula than in the breast fed infants

The latter difference was closely related to the dietary ratio of linolenate to linoleate (Table 2) It has earlier been amply demonstrated that the dietary ratio between these two fatty acids strongly influences the ratio of the fatty acids of the linoleic acid and linolenic acid series in total lipids of blood and various organs (9) and in the phosphoglycerides of brain and liver (5 25) A low dietary linolenate linoleate ratio will lead to an increase of the fatty acids of the linoleic acid series particularly of 22 5 (n−6) (25) It is evident from Table 4 which gives the fatty acid composition of plasma and red cell lecithin that the amount of 22 5 (n−6) was many times larger in formula fed than breast fed infants while the concentration of the fatty acids of the linolenic acid series in formula fed infants was only half of that in the breast fed group Recent dietary studies in the rat at this laboratory[1] have shown that the absolute amounts of linoleate and linolenate in the diets have but little effect on the ratio between the fatty acids of the linoleic and linolenic acid series of lecithin This ratio was not significantly changed when the dietary con centration of the two acids was varied between 1 to 10 calorie % as long as the ratio between the two fatty acids was kept constant On the other hand a moderate alteration of the dietary linolenate/linoleate ratio changed the ratio be tween the two fatty acid series in plasma le cithin It is thus evident that a low plasma level of fatty acids belonging to the linolenic acid series cannot be used as a proof of lack of dietary linolenate

In a previous study (17) it was shown that although the linoleic acid/arachidonic acid ratio of plasma and red blood cell lecithin in the newborns was the inverse of that in their mothers the concentration of the total poly-enoic fatty acids was equal in both and fell within the same range as that in normal adults (2) In the present study the concentration of fatty acids of the linolenic acid series was high er in the plasma and red cell lecithins of breast fed than of formula fed infants but the con centration of the total polyenoic acids was significantly lower in the breast fed than in the formula fed infants and in newborns The difference was confined to the fatty acids of the linoleic acid series The concentration of the linoleic acid in blood lecithin was about 30 lower in breast fed than in formula fed infants the difference being somewhat larger

[1] Olegård, R unpublished results

[1] Alling C Karlsson I Olegård R & Svennerholm L unpublished results

Table 5 *Fatty acid composition of red cell cephalins in breast fed and formula fed 3 month old infants*

Fatty acid	Breast fed ($n=7$)		Formula fed ($n=8$)		Difference Formula fed Breast fed	
	Mean	S D	Mean	S D	Mean	
16 0	13 7[a]	1 25	11 2	1 59	−2 5[b]	**
16 1	1 6	0 50	0 8	0 14	−0 8	***
18 0	25 1	1 94	19 2	0 87	−5 9	***
18 1	16 2	1 26	17 3	0 57	+1 1	*
18 2 (n–6)	3 5	0 32	6 0	0 47	+2 5	***
18 3 (n–3) +20 1	1 0	0 11	0 8	0 14	−0 2	*
20 3 (n–9)	traces		traces			
20 3 (n–6)	1 6	0 49	1 9	0 56	+0 3	–
20 4 (n–6)	22 6	2 45	24 6	1 81	+2 0	*
20 5 (n–3)	0 7	0 33	0 3	0 20	−0 4	**
22 4 (n–6)	3 7	0 69	8 9	1 73	+5 2	***
22 5 (n–6)	0 7	0 24	2 3	0 47	+1 6	***
22 5 (n–3)	2 0	0 33	1 6	0 64	−0 4	*
22 6 (n–3)	7 6	2 00	4 9	0 99	−2 7	**
18–22 (n–6)	32 5	2 69	43 6	3 04	+11 1	***
18–22 (n–3)	11 3	2 48	7 5	1 54	−3 8	*
(n–6) + (n–3)	43 8	3 65	51 1	2 72	+7 3	*

[a] [b] See text in Table 4

leic acid series also in red cell cephalins was 30% lower in the breast fed infants The differences in concentrations were largest for linoleic acid (18 2 ($n-6$)) 22 4 ($n-6$) and 22 5 ($n-6$) The difference in arachidonic acid (20 4 ($n-6$)) concentration was smaller and significant only at the 0 05 level

In conformity with the fatty acid patterns of plasma phosphoglycerides and red cell lecithin, the fatty acids of the linolenic acid series in red cell cephalins was 30% lower in the formula fed infants The difference was largest for 22 6 ($n-3$) The concentration of the fatty acids of the linolenic acid series was in the same range for breast fed infants as for normal adults (2)

DISCUSSION

Concentration of main lipid classes in plasma

In adults a high intake of polyenoic acids has been shown to lower plasma cholesterol levels (12) Though breast milk contains about three times as much linoleate as cow s milk formula Woodruff et al (26) found similar plasma cholesterol levels in 3 month old infants fed breast milk or the cow s milk formula The results obtained in other studies (4, 7 14, 18) of the effect of breast milk or cow s milk formulas on the plasma lipid composition during early infancy are difficult to evaluate because no information is given about the infants ages at the time of blood sampling There were also differences in e g caloric intake the type and the amount of carbohydrates and proteins as well as in the fat and linoleic acid contents which complicated comparisons of the results

Pomeranze et al (19) showed that 50 g of corn oil added daily to an infant s evaporated milk formula significantly lowered serum cholesterol levels Sweeney et al (24) found 30% lower values for plasma triglycerides cholesterol and phospholipids and lower cholesterol/phospholipid ratios in 6 week old infants fed cow s milk formulas containing 10–15 calorie % linoleic acid than in age matched infants fed the same diet but with only 0 5 calorie % of linoleic acid In a well controlled study by Lindquist & Malmcrona (13) the cholesterol level was shown to rise during the first one to two weeks after birth irrespective of the linoleic acid content of the diet The cholesterol levels afterwards continued to rise in infants on a low-linoleic acid diet (cow s milk fat, about 1 calorie-% linoleic acid) but remained constant in the infants on a high linoleic acid diet (corn oil about 18 calorie % linoleic acid)

We could not find any difference in total lipid cholesterol or phospholipid plasma concentration between the breast fed and the formula fed infants As in previous studies moderate changes in the dietary polyunsaturated fatty acids did not appear to have any significant effect on the plasma lipid levels

Although the difference in triglyceride concentration in the present study was not significant at the 0 05 level the mean triglyceride concentration was 25% higher in formula fed than in breast fed infants This difference might perhaps be explained by a higher carbo

hydrate/fat ratio in the formula than in the breast milk (1) At birth the triglyceride plasma concentration is very low and at one year of age the triglyceride level[1] is the same as in adulthood (Table 3) The 100% higher plasma triglyceride concentration of our 3 month-old infants coincided in time with the very high glucose tolerance found by v Euler et al (3) in this age group These authors suggested a relative hyperinsulinism at this age which could also explain the hypertriglyceridemia in the infants since a high correlation has been demonstrated between the insulin response to high carbohydrate diet and the degree of hypertriglyceridemia (20) in adults

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The latter difference was closely related to the dietary ratio of linolenate to linoleate (Table 2) It has earlier been amply demonstrated that the dietary ratio between these two fatty acids strongly influences the ratio of the fatty acids of the linoleic acid and linolenic acid series in total lipids of blood and various organs (9) and in the phosphoglycerides of brain and liver (5 25) A low dietary linolenate linoleate ratio will lead to an increase of the fatty acids of the linoleic acid series particularly of 22 5 $(n-6)$ (25) It is evident from Table 4 which gives the fatty acid composition of plasma and red cell lecithin that the amount of 22 5 $(n-6)$ was many times larger in formula fed than breast fed infants while the concentration of the fatty acids of the linolenic acid series in formula fed infants was only half of that in the breast fed group Recent dietary studies in the rat at this laboratory[1] have shown that the absolute amounts of linoleate and linolenate in the diets have but little effect on the ratio between the fatty acids of the linoleic and linolenic acid series of lecithin This ratio was not significantly changed when the dietary concentration of the two acids was varied between 1 to 10 calorie % as long as the ratio between the two fatty acids was kept constant On the other hand a moderate alteration of the dietary linolenate/linoleate ratio changed the ratio between the two fatty acid series in plasma lecithin It is thus evident that a low plasma level of fatty acids belonging to the linolenic acid series cannot be used as a proof of lack of dietary linolenate

In a previous study (17) it was shown that although the linoleic acid/arachidonic acid ratio of plasma and red blood cell lecithin in the newborns was the inverse of that in their mothers the concentration of the total polyenoic fatty acids was equal in both and fell within the same range as that in normal adults (2) In the present study the concentration of fatty acids of the linolenic acid series was higher in the plasma and red cell lecithins of breast-fed than of formula fed infants but the concentration of the total polyenoic acids was significantly lower in the breast fed than in the formula fed infants and in newborns The difference was confined to the fatty acids of the linoleic acid series The concentration of the linoleic acid in blood lecithin was about 30 lower in breast fed than in formula fed infants the difference being somewhat larger

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in the red cells than in the plasma Also, an other member of the linoleic acid series, namely 22 4 ($n-6$), was significantly lower in the blood phosphoglycerides of breast fed infants The absolute difference in the lecithin fraction was rather small, but was large in the blood cell cephalins

The present findings raise the question whether the low concentration of fatty acids of the linoleic acid series and the total polyenoic acids in the blood phosphoglycerides are signs of deficient dietary linoleic acid in the breast-fed infant It is widely accepted that a deficiency of essential fatty acids is reflected in an elevation of 20 3 ($n-9$) but this has not been proved in humans There were only traces of this acid in plasma and red cell phosphoglycerides of the breast fed infants We have previously stressed the importance of the homeostatic mechanism by which the level of polyenoic fatty acids of the phosphoglycerides is kept constant in the blood (17) and in the brain (23) The low concentration of total polyenoic acids in the blood phosphoglycerides suggests that this homeostatic mechanism is not fully developed or that the dietary intake of linoleic acid is insufficient in the breast fed infant during the first months of life

SUMMARY

Concentrations of the main lipid classes in plasma and the blood phosphoglyceride fatty acid patterns were measured in 3 month old infants fed breast milk or an industrial manufactured formula The levels of cholesterol and phosphoglycerides were in the normal range for young adults, but the triglyceride concentration was twice as high as in adults There were no significant differences in the levels of the plasma lipids between the two groups but the mean of the triglycerides was 25% higher in the formula fed group

The plasma and red cell phosphoglycerides had already assumed a fatty acid pattern of adult type The concentration of the total polyenoic acids was significantly lower in the breast fed group the difference being entirely confined to the fatty acids of the linoleic acid series Linoleic acid was 27% and 40%, respectively, lower in plasma phosphoglycerides and red cell lecithin of breast fed than of formula fed infants, but arachidonic acid did not show any significant difference in the two groups The concentration of the fatty acids of the linolenic acid series was higher in the breast fed infants The blood lecithin ratio between the fatty acids of the linolenic acid series and the linoleic acid series was thus much lower in the formula fed than in the breast fed infants the ratio being closely correlated with the dietary linolenate/linoleate ratio Although the concentration of essential fatty acids was low in both plasma and red cell phosphoglycerides of breast fed infants, there was no increase of 20 3 ($n-9$)

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Concentrations of the main lipid classes in plasma and the blood phosphoglyceride fatty acid patterns were measured in 3 month old infants fed breast milk or an industrial manufactured formula The levels of cholesterol and phosphoglycerides were in the normal range for young adults but the triglyceride concentration was twice as high as in adults There were no significant differences in the levels of the plasma lipids between the two groups but the mean of the triglycerides was 25% higher in the formula fed group

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two substances chosen for the determination of the glomerular filtration rate (GFR) and effective renal plasma flow (RPF) are prepared according to the dosage mentioned above in separate disposable 1 ml plastic syringes and weighed on a Mettler balance to 0 1 mg In a similar manner an "aliquot" approximating one fifth of the doses of both substances is prepared and weighed to be used as a standard Both aliquots are injected into a 500 ml volumetric flask which is then filled to the mark with tap water The two doses are injected rapidly into the clamped infusion tubing and a chronometer is started while the infusion runs fast for approximately 1 minute in order to flush both substances as quickly as possible into the blood vessels In older children the infusion is then taken away and the needle closed by its stylet while the NaCl infusion will be kept dropping as slowly as possible in younger children all through the examination in order to maintain the vein open The 4 syringes of both doses and aliquots are then weighed again and the injected amount is calculated

At regular short intervals (usually 8 16 24 3 40 55 70 and 85 min after the injection of the clearance substances) a 1.5 ml blood sample is drawn through the indwelling needle without using a tourniquet and after centrifugation 0.5 ml of plasma are pipetted into counting tubes Three samples of 500 μl of the diluted standard *solution* are prepared in the same manner (Carlsberg micropipets) All the plasma samples and the standards are measured in an Auto-Gamma (Packard Instruments International) with a 2 channel spectrometer one window being centered on the 324 kev of the photoelectric peak of ^{51}Cr the second one on the 35 kev of the peak of the ^{125}I The first channel registers only the counts of ^{51}Cr and none of ^{125}I the second window is opened in such a manner that the counts due to ^{51}Cr registered represent less than 10% of the counts counted in the first channel This ratio is recontrolled each time the channels are set for counting by determining the activity of a ^{51}Cr standard in both channels. All the samples are then counted for a period of 10 min which gives a precision of over 1% for the standard and the first plasma samples and at least 2.5% for the last ones After correction of the counts of the second channel for the ^{51}Cr activity the values of each isotope expressed in cpm/ml are plotted on semilogarithmic paper separately for ^{51}Cr EDTA and ^{125}I OIH

Calculation of the clearance

The graphic resolution of the experimental curve is performed as follows (Fig 1) a straight line is fitted through the three or four last points of the plasma disappearance curve Its intercept with the y axis is determined as well as its half life time defined as the time which passes until the radioactivity has diminished to half its original value The intercept is called A the half life time $T_{1/2a}$ The first points of the original plasma disappearance curve lie higher than the straight line the value of which is subtracted point by point from the experimentally obtained curve Thus a second straight line is obtained and its intercept B with the y axis and its half *life time* $T_{1/2b}$ are also read on the graphic The value of the clearance is then calculated using following formula

$$C = \frac{0.693 \cdot I}{A \cdot T_{1/2a} + B \cdot T_{1/2b}} \quad \text{where } I = D \frac{Wd}{Wa} Q$$

I = injected activity in cpm Wd = weight of the injected activity in g Wa = weight of the undiluted aliquot in g Q = activity of the diluted aliquot in cpm/ml D = dilution factor of the aliquot in ml

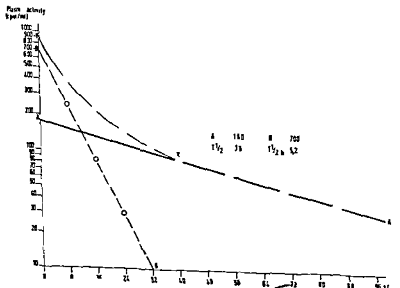


Fig 1 Resolution of the plasma disappearance curve

THE SIMULTANEOUS DETERMINATION IN CHILDREN OF GLOMERULAR FILTRATION RATE AND EFFECTIVE RENAL PLASMA FLOW BY THE SINGLE INJECTION CLEARANCE TECHNIQUE

A DONATH

From the metabolic unit of the Department of Paediatrics University of Berne Switzerland

Very shortly after Homer Smith (1) had introduced the concept of clearances into medicine, and even before its significance in physiology and hospital routine had been completely recognised, it became evident that the method was often too complicated and the procedure too cumbersome for the patient in order to enjoy a broad acceptance by the physicians Today the general opinion is unchanged and the complexity of the method still makes a large propagation impractical

This is one reason why many attempts have been made to simplify the standard technique, either by renouncing to the catheterisation of the bladder or by suppressing the intravenous infusion

In recent years, surprisingly good results have been reported using a single injection clearance technique (2–17) After 2 years of practice and over 500 clearances performed this way in adults and even more in children, it appears reasonable to us to make a review of our experience as well as of the theoretical basis of this method

METHODOLOGY

Indicator substances

The commercially available Hippuran or orthoiodohippuric acid labelled with ^{125}I (OIH ^{125}I) is used for the determination of effective renal plasma flow Its specific activity is 2 to 6 mCi/mmole of OIH ^{125}I it has been checked chromatographically for free iodine and even after 4 weeks of cold storage shows less than 2 free ^{125}I As a glomerular marker ethylendiaminotetraacetate ^{51}Cr (^{51}Cr EDTA) is used dissolved in NaCl containing Na-EDTA 0 0025 molar and with a specific activity of 40–50 mCi/mg Cr after 1 month of storage there is no free ^{51}Cr detectable Hippuran has been shown to behave as the classical paraaminohippuric acid (1) and ^{51}Cr EDTA as inulin (12 18 19) The following doses of the labelled compounds are used 40 μCi ^{125}I/m^2 and 100 μCi ^{51}Cr/m this higher dosage being due primarily to the fact that ^{51}Cr has only 12 gamma rays The photoelectric peak of ^{125}I lies at 35 kev the one of Cr at 324 kev this difference in the energy of the gamma rays allows an easy spectrometric separation of both isotopes when counted simultaneously The physical half life time of both isotopes is 60 and 27 days respectively and this allows to store them and have them available for clearance purposes at any time However no solution older than 4 weeks has ever been used

Single injection procedure

The technique described in adults elsewhere (16) has been taken over in pediatrics with very minimal changes The clearance determination is performed on hospitalized children as well as on out patients who just stay for 2 hours in the hospital A special room of the metabolic ward is devot d to these examinations which are always performed by a specialised medical and nursering team A saline infusion is started through an indwelling needle placed in a cubital vein and 1 5 ml of blood is taken which will be used as a blank in order to make sure that there is no radioactivity in the patient s blood The

stance studied each unit of time will be in case of a mamillary system (2)

$$Cl = \frac{\text{Dose}\ k_1\ k_2}{Ak_2 + Bk_1}$$ (A and B being the respective intersept $(C_1 - C_2)$ and C_2)

or by replacing the slopes by the half life time

$$Cl = \frac{\text{Dose}\ 0\ 693}{A\ T_{1/2a} + B\ T_{1/2b}}$$

In case of a catenary system where the substance must go from compartment 1 into compartment 2 before being excreted the calculation of the excretory flux is only possible if access to compartment 2 is possible as well

What is the correct model for clearances performed by the single injection technique?

Let us examine the experimental reason first Over 1 200 single injection clearances have been performed in our laboratory during the last 3 years and practically in every case it has been possible to resolve the plasma disappearance curve in two exponentials This is true for high normal clearance values (the highest GFR was 172 ml/min/1 73 m²) as for very pathological ones the lowest being 7 ml/min/1 73 m In our experience as well as in that of the other authors a 2-compartment open model cannot be disproved On the other hand the correlations obtained between clearances performed by single injection technique and by classical ones (3 5 8 9 10 12 14 16 17 18 23) indicate also that the chosen model corresponds to reality

Another proof for the mamillary system is given by our knowledge of renal physiology Even if the plasma disappearance curve does not allow to find out what are the anatomical compartments concerned renal physiology teaches us that substances which are filtered pass directly from the blood through the glomerulum and that the second compartment certainly cannot be located between the blood vessels and the glomerular barrier The same thought can be applied for the extraction of PAH or OIH from the blood by the renal tubule

These thoughts are indirect proofs or at least very strong indications that the irreversible excretion occurs from the first compartment and they most likely exclude a catenary system in which the substance studied passes from compartment 1 into compartment 2 before getting excreted This model therefore appears exact if there are really only 2 compartments

An objection often made to the single injection method is that if a patient has edema the extracellular fluid may not be only one single compartment and the system will no longer be a bicompartmental one In fact one would obtain a plasma disappearance curve composed by 3 exponentials Even if the graphic decomposition used for the determination of the individual exponentials is too rough to allow a precise separation of this third exponential the way of calculating the excretory flux is still practically correct if the model is of the mamillary type (Fig 3) In fact the value of the denominator is proportional to the surface under the disappearance curve

Assuming that this possible third compartment would only equilibrate after the 85th minute which represents the latest time the

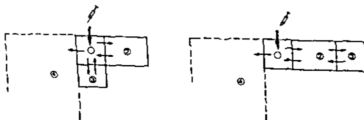


Fig 3 Open 3-compartments model

Theoretical basis of the single injection clearance

Already before the availability of radioactive substances, kinetic studies have been performed in animals and in humans but with substances which are normally not present within the organism as bromsulphalein for the study of the liver (20) If a substance is only sparsely present, an external large load will allow to procede to the determination of its volume of distribution and its clearance from the blood, and this is what Sapirstein (2) did when he injected creatinine intravenously into dogs

Tracer techniques or kinetic studies have become a very enriching and broadly spread method for clinical investigations since radio-labelled substances are available Theoretical models have been experienced in practice and a new approach is now possible, bringing up new concepts, as the measurement of calcium accretion into bone (21) or allowing a simplification of already used techniques, a simplification for the medical staff for the laboratory, and last but not least for the patient This is the reason for the enthusiastic acceptance of these tracer techniques, but also for misinterpretation and overinterpretation which led to false conclusions and often made some physicians condemn the whole concept of kinetic studies The single injection technique is based on Sapirstein s experiment (2), who measured the volumes of distribution of intravenously injected creatinine Sapirstein is very cautious in his paper and explains all the assumptions he makes Effectively there was no way to control whether they are right or not

Sapirstein proposes a 2 compartment model Today one would speak of an opened biological system of 2 compartments placed in parallel also called of a mamillary type This means that the first compartment into which the labelled substance is introduced is open and not the second one Only under such condition the mathematical treatment of Sapirstein s model is correct If the opening is in the second compartment one calls this a catenary model or a 2 compartment system in series (Fig 2) Actually the system opens into a third compartment, so huge that the labelled substance will never reach a detectable equilibrium In the mamillary model the labelled substance injected into the first compartment can either go into compartment 2 or into compartment 3 In the catenary model it must pass through compartment 2 in order to reach compartment 3 In both cases what gets into 3 will not get back, this passage is irreversible for this reason the model is called an open 2 compartment system

The mathematical treatment of a mamillary and of a catenary open 2 compartment model is similar up to a certain point but differs in some others The plasma disappearance curve is in both cases the sum of two exponentials, the concentration in the plasma at any time t is given by the formula

$$C_t = C_1 \cdot e^{-k_1 t} + C_2 \cdot e^{-k t}$$

where (C_1–C) and C_2 are the logarithms of the intercepts with the y axis and λ_1 and λ the slopes of the rapid and slow exponentials respectively

The clearance (*Cl*), which corresponds to the amount of fluid cleared from the sub

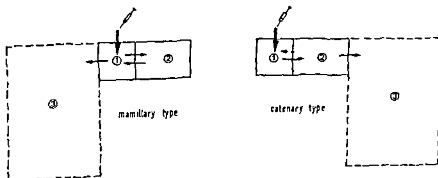


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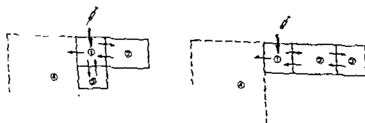


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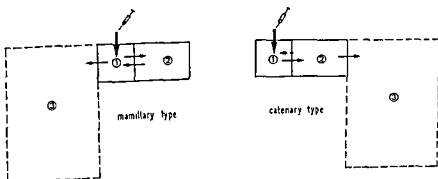


Fig 2 Open 2 compartments model

Table 1 *Single injection clearance Reproducibility*

C.D 14 years			H.G 11 years			A P 9 years		
	(ml/min/1 73 m²)			(ml/min/1 73 m²)			(ml/min/1 73 m²)	
Date	GFR	RPF	Date	GFR	RPF	Date	GFR	RPF
31 3	110	584	4 2	107	622	11 2	124	592
2.4	104	590	5.2	98	594	12.2	131	599
4 4	104	594	6 2	104	603	13.2	115	592
8 4	95	568	7 2	111	624	14.2	117	603
11 4	101	588						
Mean value	102 8	584 8		105 0	610 75		121 75	596 5
S D	5 45	10 0		5 48	13 93		7.28	5 45
S D (•)	5 30	1 71		5.22	2.26		5 96	0 92

proofs have been brought up in humans not even in normal individuals

In practice however even if the 2-compart ment open mamillary model used for calcula tion does not correspond to reality in all cases the resulting error will be so small that it lies within the limitation of any technique as com plicated as the determination of the renal clearances including the classical way of de termination

Reproducibility

In one child the renal clearances are determined by single injection technique 5 times within 12 days (Table 1) The results show a standard deviation of 5 3 for the glomerular filtra tion rate and 1 7 for the RPF In two other children the test is performed once a day 4 days in a row and shows for GFR a standard deviation of 5 5 and 5 7% respectively and for RPF 2 3 and 0 9 With a 95 % con fid nce the GFR result varies between 10 6 and 11 4 / and the RPF between 1 8 and 4 6% If one remembers that there are no basic conditions for clearance determinations it may be useless to determine the reproduci bility of clearances in one single person (27) but our results still fall within the limits of what others have found (14)

Simplified techniques

Many authors (3 8 10 13 28) despising the mathematical bases of the tracer and com partment models have oversimplified the single injection technique but as already shown in a previous paper (6) such an oversimplifica tion can only occur on the account of ac curacy In Fig 5 we have represented a case in which the ⁵¹Cr EDTA equilibrates very rapidly within the second compartment so that after 24 min both spaces are in equi librium The clearance value is indirectly pro portional to the surface under the disappear ance curve marked by *YA* (this last point lies theoretically at the infinite on the straight line *AA'*) with the simplified method which meas ures the plasma concentration after 8 and 18 min only the triangle *BB O* is delimited which obviously is much smaller than the correct sur face and therefore will give a too elevated clearance value Both the 40/60 min method and the 20/30 min method will end up with a result which will be slightly too high as the surface delimited this way underestimates the effective one On Fig 5 we have a case in which the equilibrium between compartments 1 and 2 is only obtained after 54 min The effective surface is delimited by *YA O* Again the 8/18 min method delimits the triangle *BB* O and the 20/30 min the triangle CC *O* both of them being by far too small and giv ing an overestimated renal clearance The 40/60 method on the other hand delimits the triangle *DD O* which corresponds more or less to the triangle *AA O* and only neglects the part of the surface corresponding to *YY A* From these two examples it is obvious that

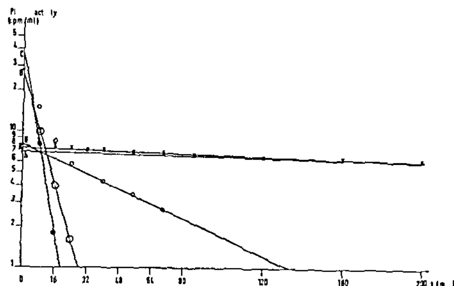


Fig 4 Plasma disappearance curve of a 3 compartments model in which a third exponential appears after the 85th minute The error by missing it is represented by the black surface between the two lines after the 180th minute *In this case it* would only make a difference of 3 ml/min on the final clearance result

plasma activity is determined with our technique, it would only cause a minimal modification of the final results of the clearance and this slight change would be a *diminution* of the clearance value, as the appearance of the third exponential would slightly increase the surface under the disappearance curve (Fig 4)

Again the model is mathematically not absolutely correct if we have a combined mamillary catenary 3 compartment system (Fig 3) where the *excretion happens from the first* compartment, but the third one is not directly related to the first one, but to the second one In such a case, the model proposed by Sapirstein is once more mathematically not absolutely exact, but the error in missing the third exponential would be so small that it *would fall within* the usual *precision* of the method If one assumes that the first compartment is plasma and the others form the extracellular fluid, such a model would appear absolutely compatible with our physiologic knowledge, showing that the extracellular fluid is not a single compartment, as it is no longer *homogenous* for the substances studied which would *first* equilibrate within a more rapidly exchangeable compartment and then within a slowlier exchangeable one, which could well be represented by edema But it is known that water (24) and substances like thiosulfate (25) or inulin (26), which equilibrate within the extracellular fluid need 4 to 8 hours to exchange completely, for this reason the single injection technique proposed, in which the plasma disappearance curve is only measured *for a duration* of 85 minutes, does not allow to reach this complete equilibration and the calculation of the size of the compartments leads to figures which do not correspond to any known anatomical space (2, 16)

All these are empirical indirect proofs of the practical exactitude of the mathematical *model The irrefutable demonstration of what* the absolutely exact model is, can only be brought by one of the following two experiments

(*a*) The theory of a bicompartimental open model teaches us that the specific activity in compartment 2 will start at 0 go up to reach maximum and then decrease again If this specific activity determined once during the decreasing period is higher than in the first compartment at the same time, the system is of the mamillary type If it is *lower it is a* catenary model

(*b*) When the specific activity in *the first* compartment and in the excreted fluid (primary urine in the case of renal clearances) are determined at the same time, the specific activity of the excreted material is lower than *the one inside compartment 1 when* the system is a catenary one they are equal if the mamillary system is correct

To our knowledge so far none of these two

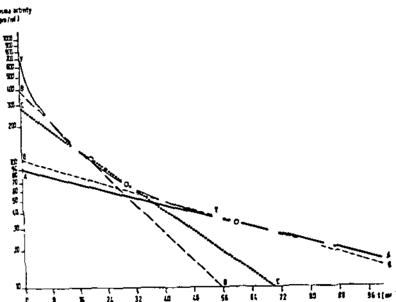


Fig 6 Bi-exponential method compared to simplified techniques II

of the indications of the determinations of glomerular filtration rate and effective renal plasma flow A more frequent determination of the kidney function in progredient renal diseases or during recovery from an acute ill ness or before and after a nephrectomy will help the pediatrician to follow his renal pa tients better the discomfort of the method is not much greater than a simple venous punc ture so that his patient and especially the boys will no longer feel reluctant to iterative clearance determinations On the other hand there are very specific indications for this single injection method which are detailed in a further paper (23)

SUMMARY

The performance of over 1 000 clearances with the single injection method allows a retrospec tive review on the value of this new technique The choice of the indicator substance the reproducibility of the method and the irradia tion dosimetry do not bring up very exciting problems The theoretical basis of the justifica tion of the single injection method has not been proven so far and the inexactitude which may happen because of the empirical assumption that the substances used behave as in an open mamillary bicompartimental model is discus sed The author comes to the conclusion that although the rightness of the model has never been proven the method will give sufficient precise results in practice and is proposed to a large extension because of its technical simplicity as well as for the patient as for the physician and the laboratory technician The irradiation dose is so small that it will prac tically never be considered as a contra indica tion

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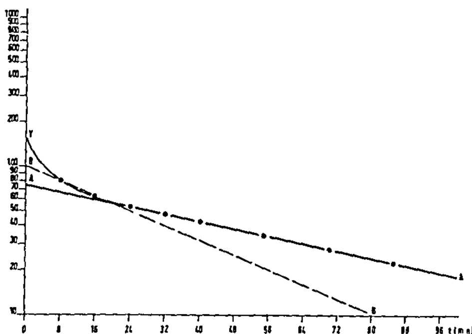


Fig 5 Bi exponential method compared to simplified techniques I

these simplified methods can be right one time or another, but that they are subject to great errors depending of the rapidity of the equilibration of compartment 1 and compartment 2 On purpose we did not mention whether these curves are obtained with ^{51}Cr EDTA or OIH-^{131}I because the same way of thinking will apply in both cases

Radiation dosimetry

The absorbed dose of both radioactive substances has been calculated according to the latest knowledge in the field of dosimetry (29), using Lowinger s (30) method of calculation We have postulated that the urine is evacuated from the bladder two hours after getting there this assumption is based on frequent controls performed during routine procedures With a normal renal function the single injection clearance method causes a total body irradiation of less than 10 mrad and in cases with a very high impairement (GFR = 10 ml/min/1 73 m²) an irradiation of less than 60 mrad These figures are valid for all ages as the dose of the radioisotope is proportional to the body surface This irradiation lies under the dosimetry of a thoracic radiography a fortunate person with a normal kidney function, who spends 3 weeks skiing in Adelboden (alt 1 500 m) rather than working in Berne (alt 500 m) will get more irradiated by cosmic rays than by the isotopes of a single injection clearance

Indications

The single injection method is particularly wellcome in the pediatric field, as especially in boys the catheterisation of the bladder always represents a psychologically dramatic procedure There is no doubt that for the patients it represents a much less discomfortable examination than the classical clearances So far the routine procedure has been in pediatrics to limit the inulin and PAH clearances to selected cases and to use the endogeneous creatinine clearance as a rough screening test for glomerular filtration rate Between the exactitude of the standard inulin and PAH clearances, which is by far not absolute and the great inaccuracy of the creatinine clearance there is a large no man s land in which the single injection clearance in our opinion appears to stay very close to the classical clearance methods Because of its technical simplicity, this single injection technique should become more and more popular and will certainly allow to extend the field

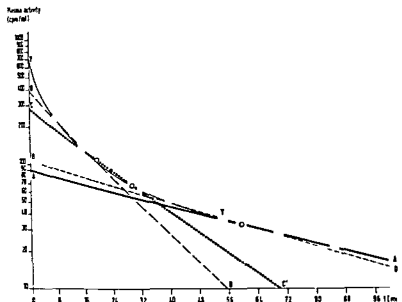


Fig 6 Bi-exponential method compared to simplified techniques II

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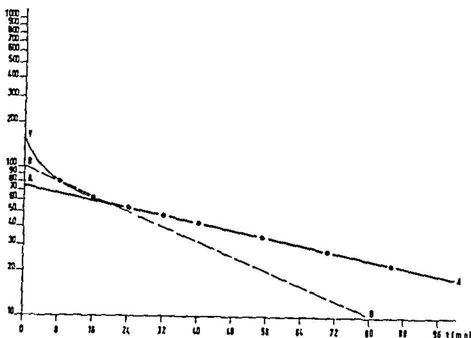


Fig 5 Bi-exponential method compared to simplified techniques I

these simplified methods can be right one time or another, but that they are subject to great errors depending of the rapidity of the equilibration of compartment 1 and compartment 2 On purpose we did not mention whether these curves are obtained with ^{51}Cr-EDTA or OIH-^{131}I because the same way of thinking will apply in both cases

Radiation dosimetry

The absorbed dose of both radioactive substances has been calculated according to the latest knowledge in the field of dosimetry (29) using Lowinger's (30) method of calculation We have postulated that the urine is evacuated from the bladder two hours after getting there this assumption is based on frequent controls performed during routine procedures With a normal renal function the single injection clearance method causes a total body irradiation of less than 10 mrad and in cases with a very high impairement (GFR = 10 ml/min/1 73 m^2) an irradiation of less than 60 mrad These figures are valid for all ages as the dose of the radioisotope is proportional to the body surface This irradiation lies under the dosimetry of a thoracic radiography a fortunate person with a normal kidney function, who spends 3 weeks skiing in Adelboden (alt 1 500 m) rather than working in Berne (alt 500 m) will get more irradiated by cosmic rays than by the isotopes of a single injection clearance

Indications

The single injection method is particularly wellcome in the pediatric field as especially in boys the catheterisation of the bladder always represents a psychologically dramatic procedure There is no doubt that for the patients it represents a much less discomfortable examination than the classical clearances So far the routine procedure has been in pediatrics to limit the inulin and PAH clearances to selected cases and to use the endogeneous creatinine clearance as a rough screening test for glomerular filtration rate Between the exactitude of the standard inulin and PAH clearances, which is by far not absolute and the great inaccuracy of the creatinine clearance there is a large no man's land in which the single injection clearance in our opinion appears to stay very close to the classical clearance methods Because of its technical simplicity, this single injection technique should become more and more popular and will certainly allow to extend the field

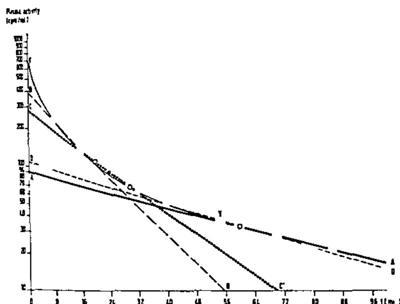


Fig 6 Bi-exponential method compared to simplified techniques II

of the indications of the determinations of glomerular filtration rate and effective renal plasma flow A more frequent determination of the kidney function in progredient renal diseases or during recovery from an acute illness or before and after a nephrectomy will help the pediatrician to follow his renal patients better the discomfort of the method is not much greater than a simple venous puncture so that his patient and especially the boys will no longer feel reluctant to iterative clearance determinations On the other hand there are very specific indications for this single injection method which are detailed in a further paper (23)

SUMMARY

The performance of over 1 000 clearances with the single injection method allows a retrospective review on the value of this new technique The choice of the indicator substance the reproducibility of the method and the irradiation dosimetry do not bring up very exciting problems The theoretical basis of the justification of the single injection method has not been proven so far and the inexactitude which may happen because of the empirical assumption that the substances used behave as in an open mamillary bicompartimental model is discussed The author comes to the conclusion that although the rightness of the model has never been proven the method will give sufficient precise results in practice and is proposed to a large extension because of its technical simplicity as well as for the patient as for the physician and the laboratory technician The irradiation dose is so small that it will practically never be considered as a contra indication

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Table 2 *Details of a study on the effect of an oral intake of ammonium chloride in a normal child*

Time (min)	Urine pH	Blood pH	Standard bicarbonate (mM/l)	Pco (mm Hg)	Total CO (mM/l)	Ammonia excretion (μEq/min/ 1 73 m b.s)
−120	Start of urine samp ing					
− 60	7 02	7 41	25 0	41	27 0	5
0	6 95	7 39	24 0	41	26 0	
	150 mEq/m b s ammonium chloride given by mouth					
60	6 37					
120	5 93	7 36	23 0	41	24 0	29
180	5 15	7 35	21 0	38	21 8	
240	4 85	7 30	18 0	35	17 9	54
300	4 82	7 29	17 0	35	17 4	54

from a typical study in a normal child is given in Table 2 The control total CO concentra tion i e before the ammonium chloride was given ranged between 23 and 30 mM/l The development of acidosis as manifested by de pression of total CO concentration had gener ally started 3 hours after the administration of ammonium chloride Five hours after the ad ministration of ammonium chloride the total CO concentration ranged between 15 and 20 mM/l in all but one child The relationship be tween total CO concentration and urine pH in normal children was characteristic A sharp fall in urine pH was noticed when the total CO was depressed from 25 to 21 mM/l When total CO_2 concentration fell beyond 18 mM/l g nerally no further fall in the urine pH was noted

The re ults from the patients with recurrent urinary tract infections and low to moderately reduced filtration rates are demonstrated in

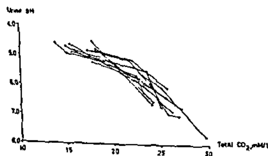


Fig 1 The relationship between blood total CO and urine pH in 11 normal children

Fig 2 and Table 3 Table 3 records a typical ex periment in a patient with low GFR (I L L) Fig 2 demonstrates the relationship between the total CO concentration and urine pH in the patients with low GFRs The total CO_2 concentration before the ammonium chloride was given ranged between 19 5 and 25 5 mM/l In 4 of the 5 patients the total CO concen tration had to be depressed well below 20 mM/l before the urine hydrogen ion secretion increased i e the urine pH was lowered In one of these patients (S N) the total CO con centration had to be depressed as low as 11 mM/l before a fall in urine pH was achieved The remaining patient did not demonstrate a fall in urine pH despite the depression of total CO concentration to 12 5 mM/l Thus 4 of 5 patients with low filtration rates demon strated principally the same relationship be- tween total CO concentration and urine pH as the normal controls but their curve was dis placed to the left i e the urine pH was de pressed at a much lower total CO concentra tion

The results from the patients with recurrent urinary tract infections and subnormal to nor- mal GFRs are summarized in Fig 3 which demonstrates the relationship between total CO concentration and urine pH Six of the patients showed the same relationship between the total CO concentration and urine pH as the normal children A further 4 of the 11 pa- tients were also able to acidify the urine but

Table 1 *Glomerular filtration rate and basal acid base data in all patients studied*

Patients	Age (years)	Sex	Clinical grouping[a]	GFR (ml/min/ 1 73 m² b s)	Serum calcium (mEq/l)	Blood pH	P_{CO} (mm Hg)	Standard bicarbonate (mM/l)
S N	11 1/12	M	B	28	4 3	7 25	44	18
K H	16 8/12	F	C	32	4 8	7 40	31	21
I L L	12 1/12	F	B	39	4 4	7 33	42	21
U K	13 0/12	M	B	50	4 8	7 40	40	25
L N	11 4/12	F	C	51	4 7	7 40	37	23
I B S	9 10/12	F	B	82	4 7	7 39	44	26
A K F	10 5/12	F	B	92	4 7	7 40	39	24
B S	9 0/12	F	B	96	5 1	7 40	39	24
E J	11 11/12	F	B	98	4 7	7 38	45	25
K O	7 0/12	F	C	104	4 8	7 42	34	23
I O	11 10/12	F	B	110	4 6	7 40	36	23
A E	14 4/12	F	C	112	4 5	7 37	41	23
M I	8 6/12	F	B	115	—	7 32	37	19
S A	9 4/12	F	B	122	—	7 37	40	23
M K	6 8/12	F	C	131	—	7 38	39	23
A K B	7 6/12	F	B	132	4 4	7 41	39	25

[a] Group A Clinical history of at most one infection with or without significant bacteriuria Group B Clinical history of one to three urinary tract infections yearly Group C Clinical history of three or more infections yearly confirmed by urine cultures or recurrent episodes of high fever and one sided abdominal pain radiating dorsally and simultaneous bacteriuria

drawn and kept anaerobically on ice pH analysis were carried out within 15 min of voiding The pH was determined on a pH meter 26 (Radiometer) Pre warmed capillary blood samples were taken and analysed for actual pH and pH in samples equili brated with 4 % and 8% CO on a pH meter 27 (Radiometer) By plotting those data on a Siggaard Andersen curve nomogram Pco standard bicarbonate concentration and blood total CO concentration could be obtained (15) In 6 normal children and 14 of the patients each urine sample was analysed for am monia by the colorimetric phenate hypochlorite method (7)

RESULTS

Table 1 demonstrates blood pH standard bi- carbonate concentration and P_{CO} from all the patients in control blood samples before am monium chloride was given The glomerular filtration rate per *1 73 m body surface dur* ring water diuresis and a clinical summary are also included The clinical classification is that used in a previous report (3) It is evident that the patients cover a wide range of filtration rates and have rather heterogeneous clinical histories There is no good relationship be- tween the frequency of urinary tract infec tions and the degree of reduction of GFR

Three of the children with recurrent urinary tract infections (S N, I L L M I) revealed laboratory signs of mild uncompensated meta bolic acidosis One of the children (K H) had *laboratory signs of compensated metabolic* acidosis In the remaining children the pH P_{CO} and standard bicarbonate concentration were within normal limits

The results of the short ammonium chloride test are best interpreted by relating the urine pH to the blood total CO concentration (4) When evaluating this relationship we have com pared the results from the normal children with those from two different groups of children with recurrent urinary tract infections namely (1) five children with low to moderately re duced *(low) filtration rates (S N K H,* I L L L N and U K) and (2) eleven chil dren with subnormal to normal (high) filtra tion rates (I B S A K F B S E J K O I O, A E M I S A M K and A K B)

The results from the normal children are demonstrated in Fig 1 and Table 2 Fig 1 shows the normal relationship between total CO concentration and urine pH A protocol

Table 4 *The maximal ammonia excretion observed The values obtained during either of the two last urine collection periods*

	Ammonia excretion (μEq/min/ 1 73 m² b s)	Ammonia excretion (μEq/100 ml glomerular filtrate)
Normal children	51±12	a
Patients with low GFRs	23±6	63±26[b]
Patients with high GFRs	45±12	44±17[b]
Normal children vs patients with low GFRs	$p<0.01$	$p>0.3$
Normal children vs patients with high GFRs	$p>0.3$	$p>0.4$
Patients with low GFRs vs patients with high GFRs	$p<0.01$	$p>0.1$

a Glomerular filtration rate not measured
b Glomerular filtration measured at previous clearance studies

fore the ammonium chloride was given All the normal children and all the patients with high filtration rates were able to respond to a depression in urinary pH with an increase in ammonia excretion All the patients with low GFRs were able to increase the ammonia excretion as the urine pH was lowered In 2 of the 5 patients however the increase was much lower than what was observed in any of the normal children and in any of the patients with high GFRs

DISCUSSION

Generally three defects of the renal acidifying mechanisms have been recognized (1) Reduced ability to reabsorb filtered bicarbonate This defect has been reported both in patients

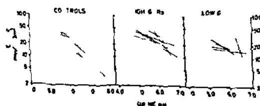


Fig 4 The relationship between urine pH and the basal and maximal ammonia excretion in 6 normal children (a) 7 patients with subnormal to normal GFRs (b) and 5 patients with low to moderately reduced GFRs (c) The scale is in lin log

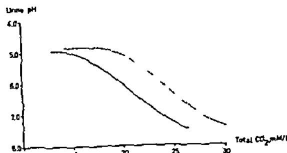


Fig 5 The theoretical relationship between blood total CO and urine pH in a normal control (--) and a patient with reduced bicarbonate threshold (—)

with generalized renal failure (16 18) and in patients with primary tubular acidosis of so-called proximal type (14) (2) An inability to establish a sufficient hydrogen ion concentration gradient between blood and tubular fluid in the distal tubule This defect has been reported mainly in patients with primary renal tubular acidosis of so-called distal type (12 6) (3) An inability of sufficient ammonia excretion This defect has mainly been reported in patients with generalized renal failure (17 10) Patients with a defect type (1) but intact "distal hydrogen ion secretion will be able to lower their urine pH once the renal threshold is passed Thus the relationship between blood bicarbonate or total CO concentration and urine pH in children with a lowered threshold value for bicarbonate is principally the same as in normal children but the curve is displaced to the left (Fig. 5) From this figure the renal bicarbonate threshold value can be roughly estimated as the total CO concentration where the urine pH starts to fall steeply Patients with a defect in distal hydrogen ion secretion are unable to increase the hydrogen ion concentration in urine i e depress the urine pH (Fig 6) Patients with defect type (3) present a reduced ammonia excretion but are generally able to acidify their urine because of a compensatory increase in excretion of titratable acids (10) All the recognized dysfunctions in the renal regulation of acid base balance should thus be discovered by the combined determination of the maximal ammonia

Table 3 *Details of a study on the effect of an oral intake of ammonium chloride in a patient with low GFR*

Time (min)	Urine pH	Blood pH	Standard bicarbonate (mM/l)	P_{CO} (mm Hg)	Total CO_2 (mM/l)	Ammonia (μEq/min/ 1 73 m² b s)
−120	*Start of urine sampling*					
− 60	6 12					
0	6 01	7 33	21 0	42	23 0	17
	150 mEq/m² b s ammonium chloride given by mouth					
60	6 01					
120	5 99					17
180	5 95	7 32	17 9	33	17 5	16
240	5 45					
300	5 19	7 26	15 5	33	15 3	19

at lower total CO_2 concentrations than that found in the normal controls One of the 11 patients reached a total CO_2 concentration of 14 2 mM/l towards the end of the test At this total CO_2 concentration the urine pH was 6 0

The maximal ammonia excretion has been evaluated statistically in Table 4 The individuals have been grouped as before, i e one group of 6 normal children one group of 5 patients with recurrent urinary tract infections and low GFRs and one group of 7 patients with recurrent urinary tract infections with high GFRs The maximal ammonia excretion in normal children averaged 51 ± 12 μEq/min/1 73 m² b s (mean ± one S D) In the children with recurrent urinary tract infections and low GFRs the maximal ammonia excretion was significantly lower than that of normal controls If, however, the maximal ammonia excretion in the patients with low GFRs is calculated per 100 ml glomerular filtrate there is no significant difference from the normal values The maximal ammonia excretion both in absolute values and calculated per 100 ml glomerular filtrate in patients with recurrent urinary tract infections and high GFRs do not differ significantly from the normal values In comparing the patients with low and high GFRs it was found that the absolute maximal ammonia excretion was significantly lower in the patients with low GFRs than in the patients with high GFRs The maximal ammonia excretion related to filtration rate was, however not significantly different in the patients with low and high filtration rates

Fig 4 demonstrates the relationship between the urine pH and the basal and maximal ammonia excretion in the children studied The basal ammonia excretion is that obtained be

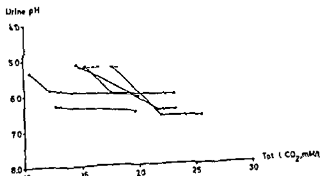


Fig 2 The relationship between blood total CO_2 and urine pH in 5 patients with low to moderately reduced filtration rates (range 28–51 ml/min/1 73 m b s) --- outer limits of the values found in normal children

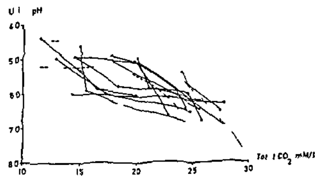


Fig 3 The relationship between blood total CO and urine pH in 11 patients with subnormal to normal filtration rates (range 82–132 ml/min/1 73 m² b s) --- outer limits of the values found in normal children

t.ly reduced filtration rates and four of the patients with subnormal to normal filtration rates demanding larger reduction in blood total CO concentration than the normal controls before a fall in the urine pH could be observed This finding was taken as a sign of reduced bi carbonate threshold It is therefore suggested that the reabsorption of filtered bicarbonate is one of the most vulnerable function of the kid ney in recurrent urinary tract infections

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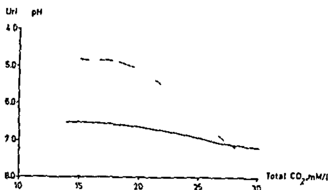


Fig 6 The theoretical relationship between blood total CO_2 and urine pH in normal controls (- -) and patients unable to excrete hydrogen ions against a gradient (—)

excretion and of the relationship between blood total CO concentration and urine pH Analysis of the relationship between total CO_2 concentration and urine pH revealed abnormalities not only in all the patients with low to moderately reduced filtration rates but also in 5 of the patients with subnormal to normal filtration rates In 4 of 5 patients with low GFRs and in 4 of the patients with high GFRs this abnormality consisted of a displacement of the relationship i e the urine was acidified but at a lower total CO_2 value than in the normal children Those children thus show signs of a reduced bicarbonate threshold High bicarbonate excretion despite low plasma bicarbonate values has previously been reported in uremic acidosis (16) The results from this study present further evidence that the renal bicarbonate threshold is reduced in renal disease, in this case recurrent urinary tract infections In fact renal bicarbonate threshold appears to be affected early in the disease process and probably before the filtration rate is reduced The present study does not allow any further speculations on the nature of the changes in hydrogen ion secretion participating in the reabsorption of filtered bicarbonate in recurrent urinary tract infections It is however, of interest that a histochemical study of carbonic anhydrase has indicated a reduced enzyme activity in renal disease (8)

Most of the patients both with high and low filtration rates were able to finally depress the urine pH to values below 5 5 (10) Thus the ability to establish a high hydrogen ion concentration in urine appeared to be intact even when the filtration rate was reduced Two patients however, one with low and one with high filtration rate were not at all able to lower urine pH during the course of the study despite the depression of total CO_2 concentration as low as 12–14 mM/l These 2 patients might thus have a defect in distal hydrogen ion secretion Very severely reduced bicarbonate thresholds with values below 14 mM/l can however, not be excluded

Studies on the maximal ammonia excretion revealed a reduction only in patients with low filtration rates When the ammonia excretion was related to the filtration rate this reduction was however no longer apparent All the patients with recurrent urinary tract infections that had subnormal to normal filtration rates demonstrated normal maximal ammonia excretion It has previously been suggested that the reduction in ammonia excretion generally found in uremic acidosis is a quantitative defect secondary to reduced nephron population (10) The ammonia excretion per functioning nephron has thus been assumed to be nearly normal A selective qualitative defect in ammonia excretion has not been demonstrated Our finding of a normal ammonia excretion filtration ratio favours the theory of a quantitative reduction of ammonia excretion in renal disease

SUMMARY

Renal control of acid base balance has been evaluated in children with recurrent urinary tract infections For reference a group of normal children has also been studied The studies were performed before and after a single oral intake of ammonium chloride The following parameters have been determined the maximal ammonia excretion and the relationship between blood total CO_2 concentration and urine pH The maximal ammonia excretion was found to be reduced in proportion to the filtration rate All the patients with low to modera-

Davidson & Sackner (16) PAH was determined according to the method of Bratton & Marshall (16)

Single injection clearance with ⁵¹Cr EDTA and ¹²⁵I OIH

Cr-ethylenediaminotetraacetate (^{51}Cr EDTA) was used as indicator substance for GFR and ^{125}Iodine marked orthoiodohippuric acid (^{125}I OIH) was used as clearance substance for determination of ERPF The dosis for the single injection clearance approximated 100 μC/m ^{51}Cr and 40 mC/m ^{125}I Each of the substance was prepared in a 1 ml disposable syringe At the beginning of the first period of simultaneous inulin and PAH-clearance they were injected into the tubing of a saline infusion that was connected to the canulized vein for 2 min in order to insure rapid and complete intravenous application of these substances The addition of saline did never exceed 10 ml Timing of the single injection clearances started with the isotope injection Two ml blood samples were drawn from the indwelling needle placed on the contralateral arm as for the inulin and PAH-clearances Nine blood samples were obtained first a control before injection then 8 16 24 32 40 55 70 and 85 min after injection of the clearance substances The radioactivity of the plasma was counted in a well type scintillation counter as described earlier (27)

Statistical evaluation

Depending on the underlying system the model of a functional linear relationship was chosen There is an exact mathematical relationship between the two obtained clearance values x representing the measurements of the single injection technique and y representing the clearance of inulin or PAH respectively Thus it was possible to determine whether the results of the single injection technique correspond to those of the inulin- and the PAH-clearance and to calculate the standard error of the regression coefficient With this model the 95 confidence limits corresponding to the inulin or PAH-clearance can be determined for a single future observed value obtained by single injection clearance The following formulas are used

regression coefficient $b_1 = \frac{\bar{y}}{\bar{x}}$

standard error of regression coefficient $s_{b_1} = \frac{s^2}{\sum_{i=1} x_i}$

whereby $(n-1)s^2 = \sum_{i=1} \left(\frac{y_i}{x_i}\right) - \frac{(\sum_{i=1}^{n} y_i)}{\sum_{i=1}^{n} x_i}$

The above mentioned 95% confidence limits for a future observed value were calculated according to the following formula

$$b\,x \pm t \sqrt{x\,s^2 + (x')^2 s_b{}^2}$$

whereas Student's $t_{\alpha, n}$ was taken from tables in the original literature (12)

Table 1 *Simultaneous determination of GFR and ERPF by means of single injection and standard clearance technique*

Patient	GFR (ml/min/1 73 m²) Inulin	^{51}Cr-EDTA	ERPF (ml/min/1 73 m²) PAH	^{125}I-OIH
1 M A	154 6	147 0	815 0	829 0
2 d P F	65 1	65 0	363 0	361 0
3 B A K.	61 9	67 0	282.0	269 0
4 C M	125 0	126 0	720 0	719 0
5 W J	49 5	56 0	221 0	227 0
6 S F	41 0	51 0	271 0	276 0
7 S R	38.2	41 0	188 0	189 0
8 d P F	28 0	37 0	209 0	211 0
9 N M	19.2	23 0	91 0	93 0
10 B A	130 0	114 0	655 0	639 0
11 L M	19 8	22.0	83 6	86 0
12 B S	142.6	136 0	852.0	830 0
13 d P F	15 5	19 0	92.3	87 0
14 K P	103 5	111 0	396 0	406 0
15 W H	149 0	143 0	798.0	780 0
16 M P	132 0	129 0	478 0	584 0
17 G C	171.0	178 0	784 0	782.0
18 W B	8 0	10 0	16 0	21 0
19 U B	103.5	101 0	596 0	589 0
20 V D	67.2	103 0	672.0	669 0
21 W T	145 6	137 0	—	—
22 D A	143 8	138 0	408 0	414 0
23 W P	106 0	96 0	516 0	557 0
24 S R	42.0	42.0	—	—
25 B A K.	114 0	114 0	491 0	551 0
26 B T	68 0	75 0	360 0	331 0
27 B I	212.0	174 0	840 0	815 0
28 S U	124 0	148 0	—	—

Glomerular filtration rate

The 28 results are shown in Table 1 in chronological order It can be seen in Fig. 1 that the inulin clearance on the ordinate and the ^{51}Cr EDTA single injection clearance on the abscissa give a regression line of the form $y = a + b_1 x$ whereby $a = 0$ i e $y = 0\,991x$ with a standard error of the regression coefficient $s_{b_1} = 0\,023$ From the fact that the regression coefficient is almost 1 and its standard error is extremely small it can be concluded that both determinations of GFR are very well in accordance *Table 2 a* in addition gives the 95 confidence limits for a single future observed ^{51}Cr EDTA value calculated from the present values with the factor $t_{0\,975} = 2\,052$

Effective renal plasma flow

25 results are shown in Table 1 In Fig 2 the PAH-clearance is plotted against ^{125}I OIH giv-

Acta Pædiat Scand 60 528–532, 1971

COMPARISON OF GLOMERULAR FILTRATION RATE AND EFFECTIVE RENAL PLASMA FLOW DETERMINATIONS OBTAINED BY A SINGLE INJECTION TECHNIQUE AND BY MEANS OF A STANDARD CLEARANCE TECHNIQUE IN CHILDREN

B VÖGELI H RIEDWYL[1] A DONATH and O OETLIKER[2]

From the renal unit and the metabolic unit of the Department of Paediatrics University of Berne Switzerland

Single injection techniques to determine glomerular filtration rate and effective renal plasma flow from plasma disappearance curves without urine collections, have repeatedly been studied (1, 2 3, 8 9 11, 13, 14 15, 17, 18, 21–25) One of the reasons to eventually introduce the single injection method is its simplicity without need of collecting urine The single injection technique for determination of glomerular filtration rate with ^{51}Cr-EDTA and of effective renal plasma flow with ^{131}I-OIH has been introduced in adult patients in a number of clinics (18 22) Since further empirical evaluation of the theoretical model (17) is strongly suggested by Truniger et al (22) it was decided to compare the single injection technique, as proposed for children by Donath (4), with a standard clearance technique The comparison was done by simultaneous determination of glomerular filtration rate and effective renal plasma flow by the two different methods

[1] Institut fur mathematische Statistik und Versicherungslehre PD Dr H Riedwyl Leiter Prof Dr W Wegmuller

[2] Supported by grant 5311 3 of the Swiss National Foundation for Scientific Research

MATERIAL AND METHODS

Selection of patients

All boys and girls needing determination of renal function were taken into the study This comprised infants as well as children up to the age of 16 years Their functional status ranged from severe insufficiency to high normal values Only those results of inulin and PAH clearance were accepted for statistical evaluation in which clearances of each period did not differ more than 20 ml/min/1 73 m² The results obtained by the single injection technique were only accepted for evaluation when technical errors had been excluded An independent investigator performed the statistical comparison 28 determinations of glomerular filtration rate (GFR) and 25 determinations of effective renal plasma flow (ERPF) will be compared in this study

Inulin and PAH clearance

After a priming dose of 1 ml/kg 10% inulin and 0 05 ml/kg 20 % PAH solution an intravenous sustaining infusion was started containing enough inulin and PAH to maintain a plasma inulin level of 50 mg/100 ml and a PAH level of 2 mg/100 ml The infusion rate was 1 ml/min/m² After an equilibration period of 45 min three clearance periods of 30 min duration were taken Repeated venous blood samples were obtained from an indwelling needle that was flushed with a slightly heparinized isotonic saline solution after each sampling

Except in three patients where catheterisation of the bladder was not necessary the urines were obtained through an indwelling urethral catheter Inulin was determined according to the anthron method of

Davidson & Sackner (16) PAH was determined according to the method of Bratton & Marshall (16)

Single injection clearance with ⁵¹Cr EDTA and ¹²⁵I OIH

Cr-ethylenediaminotetraacetate (^{51}Cr EDTA) was used as indicator substance for GFR and ^{125}Iodine marked orthoiodohippuric acid (^{125}I OIH) was used as clearance substance for determination of ERPF The dosis for the single injection clearance approximated 100 μC/m ^{51}Cr and 40 mC/m ^{125}I Each of the substance was prepared in a 1 ml disposable syringe At the beginning of the first period of simultaneous inulin and PAH-clearance they were injected into the tubing of a saline infusion that was connected to the canulized vein for 2 min in order to insure rapid and complete intravenous application of these substances The addition of saline did never exceed 10 ml Timing of the single injection clearances started with the isotope injection Two ml blood samples were drawn from the indwelling needle placed on the contralateral arm as for the inulin and PAH-clearances Nine blood samples were obtained first a control before inj ction then 8 16 24 32 40 55 70 and 85 min after injection of the clearance substances The radioactvity of the plasma was counted in a well type scintillation counter as described earlier (22)

Statistical evaluation

Depending on the underlying system the model of a functional linear relationship was chosen There is an exact mathematical relationship between the two obtained clearance values x representing the measurements of the single injection technique and y representing the clearance of inulin or PAH respectively Thus it was possible to determine whether the results of the single injection technique correspond to those of the inulin and the PAH clearance and to calculate the standard error of the regression coefficient. With this model the 95% confidence limits corresponding to the inulin or PAH clearance can be determined for a single future observed value obtained by single injection clearance The following formulas are used

regression co fficient $b_1 = \frac{y}{x}$

standard error of regression coefficient $s_b = \frac{s^2}{\sum_{i=1} x_i}$

whereby $(n-1)s = \sum_{i=1} \left(\frac{y_i}{x_i}\right) - \frac{(\sum_{i=1} y_i)^2}{\sum_{i=1}^{n} x_i}$

The above mentioned 95% confidence limits for a future observed value were calculated according to the following formula

$b_1 x \pm t \sqrt{x s + (x')^2 s_b^2}$

whereas Student s t_{97} was taken from tables in the original literature (12)

Table 1 *Simultaneous determination of GFR and ERPF by means of single injection and standard clearance technique*

	GFR (ml/min/1 73 m²)		ERPF (ml/min/1 73 m²)	
Patient	Inulin	Cr-EDTA	PAH	¹³¹ I-OIH
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2. d P F	65 1	65 0	363 0	361 0
3 B A K	61 9	67 0	282 0	269 0
4 C M	125 0	126 0	720 0	719 0
5 W J	49 5	56 0	221 0	227 0
6 S F	41 0	51 0	271 0	276 0
7 S R.	38 2	41 0	188 0	189 0
8 d P F	28 0	37 0	209 0	211 0
9 N M	19.2	23 0	91 0	93 0
10 B A	130 0	114 0	655 0	639 0
11 L M	19 8	22 0	83 6	86 0
12. B S	142 6	136 0	852 0	830 0
13 d P F	15 5	19 0	92 3	87 0
14 K P	103 5	111 0	396 0	406 0
15 W H	149 0	143 0	798 0	780 0
16 M P	132.0	129 0	578 0	584 0
17 C C.	171 0	178 0	784 0	782.0
18 W B	8 0	10 0	16 0	21 0
19 U B	103 5	101 0	596 0	589 0
20 V D	67 2	103 0	672 0	669 0
21 W T	145 6	137 0	—	—
22 D A	143 8	138 0	408 0	414 0
23 W P	106 0	96 0	516 0	557 0
24 S R	42.0	42.0	—	—
25 B A K	114 0	114 0	591 0	551 0
26 B T	68 0	75 0	360 0	331 0
27 B I	212.0	174 0	840 0	815 0
28 S U	124 0	148 0	—	—

Glomerular filtration rate

The 28 results are shown in Table 1 in chronological order It can be seen in Fig 1 that the inulin clearance on the ordinate and the ¹Cr EDTA single injection clearance on the abscissa give a regression line of the form $y = a + b_1 x$ whereby $a = 0$ i e $y = 0\,991x$ with a standard error of the regression coefficient $s_{b_1} = 0\,023$ From the fact that the regression coefficient is almost 1 and its standard error is extremely small it can be concluded that both determinations of GFR are very well in accordance Table 2 *a* in addition gives the 95 confidence limits for a single future observed ^{51}Cr EDTA value calculated from the present values with the factor $t_{0\,975} = 2\,052$

Effective renal plasma flow

25 results are shown in Table 1 In Fig 2 the PAH-clearance is plotted against ¹ ⁵I OIH giv

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COMPARISON OF GLOMERULAR FILTRATION RATE AND EFFECTIVE RENAL PLASMA FLOW DETERMINATIONS OBTAINED BY A SINGLE INJECTION TECHNIQUE AND BY MEANS OF A STANDARD CLEARANCE TECHNIQUE IN CHILDREN

B VÖGELI H RIEDWYL[1] A DONATH and O OETLIKER[2]

From the renal unit and the metabolic unit of the Department of Paediatrics University of Berne Switzerland

Single injection techniques to determine glomerular filtration rate and effective renal plasma flow from plasma disappearance curves without urine collections, have repeatedly been studied (1 2, 3, 8 9, 11, 13, 14, 15, 17, 18, 21–25) One of the reasons to eventually introduce the single injection method is its simplicity without need of collecting urine The single injection technique for determination of glomerular filtration rate with ^{51}Cr EDTA and of effective renal plasma flow with ^{125}I-OIH has been introduced in adult patients in a number of clinics (18 22) Since further empirical evaluation of the theoretical model (17) is strongly suggested by Truniger et al (22) it was decided to compare the single injection technique as proposed for children by Donath (4), with a standard clearance technique The comparison was done by simultaneous determination of glomerular filtration rate and effective renal plasma flow by the two different methods

[1] Institut fur mathematische Statistik und Versicherungslehre PD Dr H Riedwyl Leiter Prof Dr W Wegmüller

[2] Supported by grant 5311 3 of the Swiss National Foundation for Scientific Research

MATERIAL AND METHODS

Selection of patients

All boys and girls needing determination of renal function were taken into the study This comprised infants as well as children up to the age of 16 years Their functional status ranged from severe insufficiency to high normal values Only those results of inulin and PAH clearance were accepted for statistical evaluation in which clearances of each period did not differ more than 20 ml/min/1 73 m² The results obtained by the single injection technique were only accepted for evaluation when technical errors had been excluded An independent investigator performed the statistical comparison 28 determinations of glomerular filtration rate (GFR) and 25 determinations of effective renal plasma flow (ERPF) will be compared in this study

Inulin and PAH clearance

After a priming dose of 1 ml/kg 10 inulin and 0 05 ml/kg 20% PAH solution an intravenous sustaining infusion was started containing enough inulin and PAH to maintain a plasma inulin level of 50 mg/100 ml and a PAH level of 2 mg/100 ml The infusion rate was 1 ml/min/m² After an equilibration period of 45 min three clearance periods of 30 min duration were taken Repeated venous blood samples were obtained from an indwelling needle that was flushed with a slightly heparinized isotonic saline solution after each sampling

Except in three patients where catheterisation of the bladder was not necessary the urines were obtained through an indwelling urethral catheter Inulin was determined according to the anthron method of

inulin and PAH clearances that the isotope method can be regarded to be sufficiently ac curate for all practical purposes

The seemingly wide range of the confidence limits for a single future value by means of the single injection technique is due to the rela tively small number of cases Since the regres sion coefficient for GFR as well as for ERPF is close to 1 and its standard error is extremely small the conclusion of this study is not ques tioned by this fact

The reference value of the classical clear ance technique with continuous intravenous in fusion of the clearance substance and urine col lection by means of bladder catheterisation is obviously not questioned by this report This method is however often criticised for its in convenience for the child and moreover for the risks of bladder catheterisation (5 15 18 20 22 25 26) In addition the chemical determination of inulin and PAH may sig nificantly be altered by the presence of other substances like medicaments (16) The vari ability of the inulin and PAH-clearances may be as high as 10 % Zender et al (26) at tribute this mainly to the difficulty of collect ing urines appropriately Nevertheless for ex act and physiologic studies with determination of special functional parameters the use of the classical clearance methods with urine collec tion is furtheron indispensable

If on the other hand only GFR and ef fective renal plasma flow are asked for the use of the single injection method has its value No catheterisation of the bladder is necessary Generally only one vein needs to be punctured The danger of irradiation is minimal because of the very short biological halftime of the substances It is less than 60 min for ^{51}Cr EDTA and less than 30 min for ^{1}I OIH when renal function is normal and less than 6 hours and 100 min respectively in advanced renal failure Thus the total irradia tion dose is far lower than that of an intra venous urography This view is well in agree ment with the opinion of others (10) The following situations are considered as special indications for the use of the single injection technique in pediatrics

(*a*) impossibility of an appropriate collec tion of urine as in patients with severe oliguria in patients with Bricker bladder or ureterosig-moidostomy and in patients with hydronephro-sis or vesico-ureteral reflux

(*b*) in patients with increased risk of infec tion by catheterisation for instance after uni-lateral nephrectomy

SUMMARY

In children 28 values for glomerular filtra-tion rate and 25 values for effective renal plasma flow were simultaneously obtained by means of the inulin and PAH-clearance and by means of a single injection method with ^{51}Cr EDTA and ^{1} I OIH These were com pared in an appropriate statistical model The results show good agreement of the two meth-ods The single injection method can there fore be of value in childhood when only val-ues for GFR and effective renal plasma flow are asked or especially if inappropriate urinary collection is expected or bladder catherisa tion is contraindicated It has no place in stud ies concerned with renal physiology or patho-physiology

ACKNOWLEDGEMENTS

The authors wish to thank Miss K Geleva Miss R Kusic and Miss S Schultz for their most valid technical assistance

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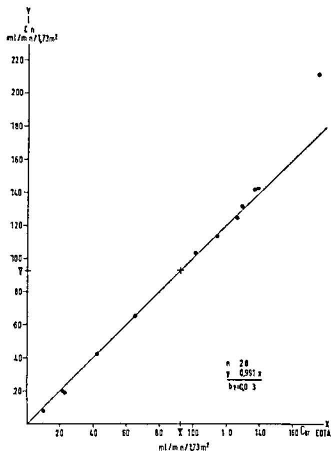


Fig 1 Relation $C_{^{51}Cr\ EDTA}-C_{Inulin}$

ing the regression line $y = 1.007x$ standard error $s_b = 0.007$ The correlation of the two methods is excellent The 95% confidence limits of a future observed [1] I-OIH value are shown in Table 2 *b* Factor $t_{0.97} = 2.064$

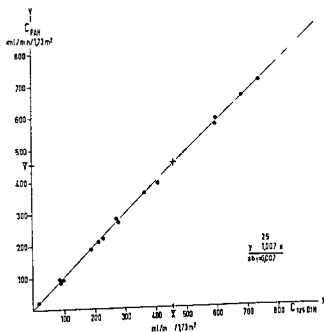


Fig 2 Relation $C_{^{125}I\text{-}OIH}-C_{PAH}$

Table 2 *Calculation of the 95% confidence limits for a single future observed value*

(*a*) *GFR* x = ⁵¹Cr EDTA y = Inulin		
if x = 10 ml/min	17 67 > y > 2 16 ml/min	
20	30 80	8 84
40	55 13	24 17
60	78 64	40 29
80	101 52	57 07
100	124 06	74 18
120	146 36	91 52
140	168 44	108 08
160	190 48	126 69
180	212 25	146 57
(*b*) *ERPF* x = ¹²⁵I OIH y = PAH		
if x = 50 ml/min	61 25 > y > 39 48 ml/min	
100	116 15	85 31
200	223 36	179 56
300	329 13	275 25
400	434 30	371 60
500	538 73	468 57
600	642 97	565 79
700	746 97	663 25
800	850 78	760 91

DISCUSSION

The observation that the regression line passes through the origine ($a = 0$) is consistent with the view of earlier investigators (6, 7, 9, 22) that [1]Cr EDTA is handled by the kidneys like inulin They contrast however to some extent with the findings of Stacy et al (19) who considers a possible ⁵¹Cr EDTA chelate binding to plasma proteins This has been refused however by Favre et al (6) Speck (18) has observed that [1]Cr-EDTA measurements underestimate GFR. The present results are in accordance with this view to some extent in the high normal range (Fig 1), but not in normal and low values

In order to determine effective renal plasma flow it was crucial to maintain a plasma level around 2 mg/100 ml PAH, since renal extraction of PAH would be incomplete with plasma levels above 3 mg/100 ml (20) This was the reason that three determinations of PAH clearance had to be excluded from the ERPF study (Table 1)

The statistical results show such good correlation between the single injection method for determination of GFR and ERPF with the

inulin and PAH-clearances that the isotope method can be regarded to be sufficiently accurate for all practical purposes

The seemingly wide range of the confidence limits for a single future value by means of the single injection technique is due to the relatively small number of cases Since the regression coefficient for GFR as well as for ERPF is close to 1 and its standard error is extremely small the conclusion of this study is not questioned by this fact

The reference value of the classical clearance technique with continuous intravenous infusion of the clearance substance and urine collection by means of bladder catheterisation is obviously not questioned by this report This method is however often criticised for its inconvenience for the child and moreover for the risks of bladder catheterisation (5 15 18 20 22 25 26) In addition the chemical determination of inulin and PAH may significantly be altered by the presence of other substances like medicaments (16) The variability of the inulin and PAH-clearances may be as high as 10% Zender et al (26) attribute this mainly to the difficulty of collecting urines appropriately Nevertheless for exact and physiologic studies with determination of special functional parameters the use of the classical clearance methods with urine collection is furtheron indispensable

If on the other hand only GFR and effective renal plasma flow are asked for the use of the single injection method has its value No catheterisation of the bladder is necessary Generally only one vein needs to be punctured The danger of irradiation is minimal because of the very short biological halftime of the substances It is less than 60 min for ^{51}Cr EDTA and less than 30 min for ^{131}I OIH when renal function is normal and less than 6 hours and 100 min respectively in advanced renal failure Thus the total irradiation dose is far lower than that of an intravenous urography This view is well in agreement with the opinion of others (10) The following situations are considered as special indications for the use of the single injection technique in pediatrics

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SUMMARY

In children 28 values for glomerular filtration rate and 25 values for effective renal plasma flow were simultaneously obtained by means of the inulin and PAH-clearance and by means of a single injection method with ^{51}Cr EDTA and ^{131}I OIH These were compared in an appropriate statistical model The results show good agreement of the two methods The single injection method can therefore be of value in childhood when only values for GFR and effective renal plasma flow are asked or especially if inappropriate urinary collection is expected or bladder catheterisation is contraindicated It has no place in studies concerned with renal physiology or pathophysiology

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MALNUTRITION AND SIZE OF THE CEREBRAL VENTRICLES[1]

Echoencephalographic Studies in Infants and Young Children
Preliminary Communication

B VAHLQUIST G ENGSNER and IRENE SJOGREN

From the Department of Paediatrics University Hospital Uppsala Sweden and the Ethiopian Nutrition Institute Addis Ababa Ethiopia

Severe caloric undernutrition and/or severe protein malnutrition instigated at an early age may cause permanent damage to the brain thereby affecting weight cell number and fine structure as well as biochemistry and function as has been clearly shown from animal experiments (3 8 17 18)

Clinical observations rarely lend themselves to such easy interpretation as well planned animal experiments Malnutrition at an early age in children of developing countries is often complex in origin (intra and extrauterine influence simultaneous nutritient depletion and infection other ecological factors) Furthermore the possibilities of analysing the human brain with respect to structure and biochemistry are hampered by the fact that biopsy on living subjects cannot be performed Much of our available knowledge stems from autopsy findings in children dying at varying ages and for reasons of varying complexity In one respect however follow up studies of human beings who have experienced severe early malnutrition should lend themselves to more detailed interpretation than is possible in animals namely when dealing with complex brain functions as reflected in mental behaviour and intelligence But here again evaluation of the effect of a deficient nutrient supply *per se* becomes extremely difficult (2)

It seems obvious that methods which permit an *in vivo* appreciation of the status of the human brain and which allow repeated examinations of a longitudinal character in individual children deserve special interest Under this heading it has so far been possible to include only head circumference (as an indirect measure of brain volume (13 14)) and transillumination (as an expression of subarachnoidal effusion (6))

In the following another type of study will be described namely echoencephalography This method makes possible a determination of the size of the ventricular cavities of the brain and this in combination with the head circumference and transillumination provides more comprehensive information on the brain volume in vivo

The studies presented in this paper were carried out at the Ethiopian Nutrition Institute Addis Ababa This institute is supported by the Ethiopian government and the Swedish International Development Authority (SIDA) (15)

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[1] CNU Report no 5.
This work is dedicated to Professor K H Schafer Pediatric Clinic Eppendorf Krankenhaus Hamburg on his 60th birthday

CLINICAL MATERIAL

A total of 66 Ethiopian infants and children 29 girls and 37 boys of ages 2 weeks to 29 months were examined (Fig 1)

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RESULTS

Weight in relation to age

As can be seen in Fig 4 the body weights in relation to age of all the controls up to 9 months of age lay within—or on the borderline of—the normal ranges (mean ±2 S D) for Swedish infants (5) In the control children

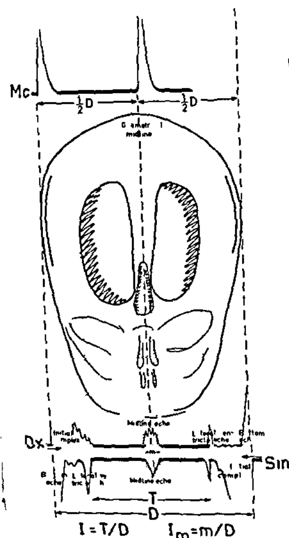


Fig 2 Schematic pneumogram compared with enlarged schematic echoventriculogram (EVG see Fig 3) where *Mc* is the midline control *Dx* the echoencephalogram from the right and *Sin* an inverted echoencephalogram from the left temporal region. The position of the lateral ventricle echoes correspond to the lateral surface of the lateral ventricles, and the echo-free zones to the widths of the lateral ventricles

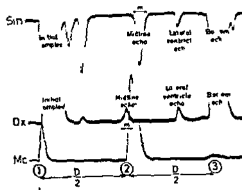


Fig 3 An echoventriculogram (EVG) within the grey field where *Mc* represents the midline control *Dx* an echoencephalogram from the right and *Sin* one from the left temporal region The graphical representation and index calculation is explained in the text

above 9 months of age the weights lay in the lower part of or somewhat below the normal range for Swedish children

All the marasmic and kwashiorkor patients showed weights far below the normal range with one exception a 7 month old boy with a disproportionately large head he was also the only one of the marasmic patients who had a lateral ventricle index somewhat above the normal range [1]

Head circumference in relation to age

Fig 5 shows that 35 of the 38 control children had a head circumference within the normal range according to O Neill (7) and Westropp & Barber (16) Only 2 children showed values slightly below the lower range and one girl of 19 months of age had a head circumference of 54 cm

[1] This boy developed all classical signs of infantile hydrocephalus during a 2 month follow up period

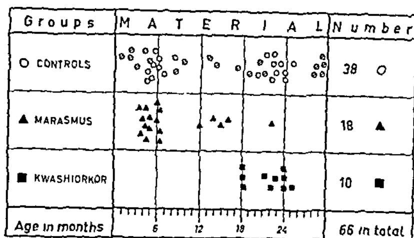


Fig 1 Age distribution of children examined

Control group

This group consisted of 38 children (18 girls and 20 boys) who were apparently healthy and had no history of malnutrition or severe disease Of these 19 children (8 girls and 11 boys) between 2 weeks and 29 months of age were residents at the Children s Home (*marked Ø in the figures*) *an orphanage attached to* the Ethiopian Nutrition Institute and 19 (10 girls and 9 boys) between 4 and 26 months of age belonged to well to do and otherwise privileged Ethiopian families (marked O in the figures) The children at the orphanage were all examined 2 or 3 times *Only the* values from the 1st examination are presented

Marasmic group

Eighteen patients 7 girls and 11 boys from 3 5 to 22 months of age with *confirmed nutritional maras*mus were attending either a special Nutrition Rehabilitation Clinic run at the Lidetta MCH Centre (14 children 6 girls and 8 boys) or the Nutrition Rehabilitation Clinic run by the Ethio Swedish Pediatric Clinic (4 children 1 girl and 3 boys) The children were examined twice with an interval of about 1 month Only values from the last examination are *presented*

Kwashiorkor group

Ten patients 4 girls and 6 boys between 18 and 26 months of age were admitted to the Ethio Swedish Pediatric Clinic where the diagnosis of kwashiorkor was confirmed The children in this group were examined twice first between 1 and 2 weeks after admission—at this time all children were free from visible oedema—and secondly after further 2 weeks In this case only the values from the first examination are presented

METHODS

All the children included in the study were examined by the same pediatrician (G E) Standard anthropometric data including body weight and head circumference were recorded In addition the widths of the lateral ventricles of the brain were measured by echoencephalography according to the method of Sjogren (10)

The echoencephalograph used was a Siemens apparatus with a camera (Polaroid Land) for instant recording of the oscillographic tracings The probe used *in this study had a frequency of 2 megacycles/sec* and a diameter of 24 mm Liquid paraffin was used as contact medium between the head and the probe The head was not shaved prior to the examination

The probe was held in contact with the scalp a few centimetres above and slightly anterior to the external auditory meatus The tracing on the oscilloscope was studied while the probes were moved Pictures were taken when the midline echo and the lateral ventricle echo on the opposite side were of maximal steepness on the true axis (Fig 2) The amplification was then increased and the distribution of the parenchymal echoes was noted

Interpretation of the echoencephalograms and index calculations from the echoventriculogram

An echoventriculogram (EVG) consists of a midline control (*Mc*) and echoencephalograms from the right (*Dx*) and left (*Sin*) sides of the head Fig 3 illustrates a typical echoventriculogram and diagram of the index calculation

*The geometrical midline of the head and the ex*ternal diameter (*D*) in the frontal plane were obtained from the midline control study From each echoencephalogram the distances from the initial complex to the midline echo the contralateral ventricle echo and the bottom echo were measured These distances were measured and marked in the figure from left to right for the *Dx* echoencephalograms and from right *to left for the Sin echoencephalogram*

The lateral ventricle index (*I*) expresses the relation between the transverse width (*T*) of the two lateral ventricles and the external diamet*er (D) of the child s* head i e *T/D* Normally this index should not exceed 0 33 (or 33% of the external diameter of the head) in newborn babies In children of 1 year of age or more the lateral ventricle index should not exceed 0 29

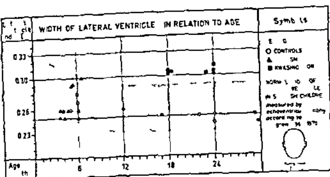


Fig 6 Width of lateral ventricles in relation to age

brain weights at autopsy were found to be normal for the age In contrast to these findings Monckeberg (6) observed increased transillumination in infants with marasmus pointing to more pronounced stunting of the brain growth than was indicated by head circumference measurements alone in the same patients

In children dying with a disease picture of severe marasmus Winick & Rosso (19) found a close correlation between head circumference and DNA content of the brain thus indicating *that the* head circumference may be a good index of the total *number of brain cells* The number of children examined was limited however

In view of the conflicting reports it seemed important to scrutinize further the relationship between head circumference and brain size An increase in volume of the cav*ities in the interior* of the brain and/or of the fluid layer on the exterior of the brain will d*iminish the* size of the brain substance in relation to the head circumference The same is true *for an* increase in cranial thickness (soft tissue included) A diminution of the inner cavities and/or a reduction of the skull thickness will have the opposite effect.

Echoencephalography makes it poss*ible to* perform accurate and repeated determinations of the widths of the lateral ventricles with no discomfort or harm to the child A comparison of the findings in echoencephalography with those of pneumo-encep*halography* has shown a good correlation (11) The present study showed in the children with kwashiorkor examined 1–2 weeks after admission when oedema was no longer visible a slight but significant increase of the widths of the lateral ventricles This deviation from the normal was still found to be present to the same extent at reexamination 2 weeks later The children with marasmus on the other hand showed values within the normal range when examined twice with an interval of 1 month

Are there any sources of error which might render invalid the interpretation of these results when comparing them with those obtained in healthy children? Transillumination studies in our series so far performed only in children with kwashiorkor showed moderately but definitely increased values However even if these latter findings can be interpreted in the usual way as an expression of increased fluid between the brain surface and the skull (which is perhaps not truly valid until the influence of bone thickness and structure and of soft tissue oedema etc has been further elucidated) this will not compromise the finding of an increased T/D value in kwashiorkor children as an expression of relatively increased ventricular widths

One source of error in studies in a developing country relates to difficulties in obtaining an exact age For children in the first few years of life the error is usually not great and furthermore the ventricular index as seen in Fig 6 does not change markedly with age after the first 6 months of life

The reason why the marasmic infants and children showed a normal ventricular index whereas those with kwashiorkor showed an in

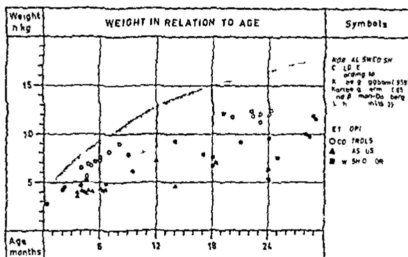


Fig 4 Weight in relation to age

Eleven of the 18 patients with marasmus had a head circumference below the normal range. The remaining 7 of the patients with marasmus and all 10 of those with kwashiorkor had a head circumference within the lower part of the normal range.

Width of lateral ventricles in relation to age

As can be seen in Fig 6 none of the controls and only one of the marasmic patients (see above) had a lateral ventricle index above the normal range according to Sjögren (12).

All 10 patients with kwashiorkor had higher lateral ventricle indices than the controls of corresponding ages which meant that their lateral ventricles were wider than normal. This difference is statistically significant ($p < 0.01$).

DISCUSSION

Children dying with a disease picture of severe malnutrition exhibit a brain weight which is definitely below the normal for height as well as for age (1).

In children surviving a period of early, severe malnutrition the head circumference has been found to lag significantly behind (13, 14). The validity of this latter study and especially the interpretation of the findings have been challenged, however (4).

The head circumference does not necessarily reflect the brain size. Robinov (9) examined 16 children who died of congenital heart disease and whose growth had been severely impaired since birth. Their head circumferences were 8.2% below the mean for their age but their

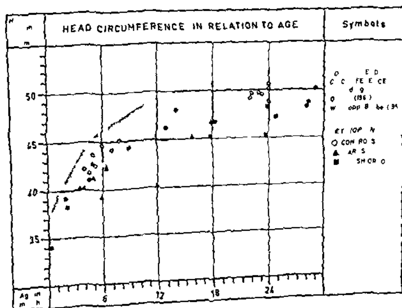


Fig 5 Head circumference in relation to age

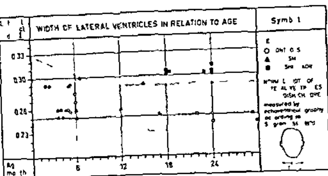


Fig 6 Width of lateral ventricles in relation to age

brain weights at autopsy were found to be normal for the age In contrast to these findings Monckeberg (6) observed increased transillumination in infants with marasmus pointing to more pronounced stunting of the brain growth than was indicated by head circumference measurements alone in the same patients

In children dying with a disease picture of severe marasmus Winick & Rosso (19) found a close correlation between head circumference and DNA content of the brain thus indicating that the head circumference may be a good index of the total number of brain cells The number of children examined was limited however

In view of the conflicting reports it seemed important to scrutinize further the relationship between head circumference and brain size An increase in volume of the cavities in the interior of the brain and/or of the fluid layer on the exterior of the brain will diminish the size of the brain substance in relation to the head circumference The same is true for an increase in cranial thickness (soft tissue included) A diminution of the inner cavities and/or a reduction of the skull thickness will have the opposite effect

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One source of error in studies in a developing country relates to difficulties in obtaining an exact age For children in the first few years of life the error is usually not great and furthermore the ventricular index as seen in Fig. 6 does not change markedly with age after the first 6 months of life

The reason why the marasmic infants and children showed a normal ventricular index whereas those with kwashiorkor showed an in

crease is not clear It cannot be explained by the difference in age alone, since the findings in the marasmic children of similar age to those with kwashiorkor were the same as in the young infants with marasmus

The body weight of the kwashiorkor children was in most cases markedly below the lower normal limit This might indicate that a prolonged period of protein calorie malnutrition had preceded the onset of the kwashiorkor symptoms

Further studies with repeated examination of the ventricular index in individual children will give an answer to the question of whether the increased ventricular index observed in children with kwashiorkor up to 3–4 weeks after initiation of treatment is a temporary phenomenon or not Such studies are now under way

They also include parallel transillumination examinations and studies of the nerve conduction velocity, as well as scorings of the psycho social and motor ages of the children

SUMMARY

Examination of the size of the cerebral ventricles by means of echoencephalography in Ethiopian infants and young children with severe malnutrition showed a moderate but significant increase in children with kwashiorkor, examined up to 3–4 weeks after admission whereas children with marasmus showed no deviation from the normal

Further studies will show whether or not this abnormality in the kwashiorkor children is a transient phenomenon The observation gives one more reason for caution in using head circumference as a measure of brain size

ACKNOWLEDGEMENT

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HIGH FREQUENCY OF SALMONELLA SPECIES AS A CAUSE OF NEONATAL MENINGITIS IN IBADAN, NIGERIA

A Review of Thirty eight Cases

NAOMI BARCLAY

From the Department of Paediatrics University College Hospital Ibadan Nigeria

It is well known that gram negative enterobacteria account for a substantial proportion of the cases of meningitis occurring in early infancy (13, 16, 23) However, apart from three extensive reviews in 1946 1951, 1958 there has been a notable silence on cases of men ingitis due to salmonella species, and since 1958 only 33 cases have been reported

Analysis of cases of infantile meningitis in Ibadan showed a high incidence of salmonella infections, many of which were resistant to the standard antibiotics

PATIENTS AND METHODS

All cases of salmonella meningitis admitted to the University College Hospital Ibadan between Janu ary 1961 and May 1969 were reviewed 508 other cases of bacterial meningitis (excluding tuberculous) admitted between January 1964 and May 1969 were studied for comparison Nine patients with abnor malities of the central nervous system were ex cluded An analysis of the pattern of antibiotic sen sitivity of salmonella isolated from blood cultures over the same period was also made

Once a diagnosis of meningitis was decided on all the children received penicillin (20 000 units/kg/24 h) and chloramphenicol (50–100 mg/kg/24 h) usually intravenously with the addition of sulphadiazine (200 mg/kg/24 h) by the same route except in neonatal or dehydrated patients No intrathecal antibiotics were administered Therapy was modified as necessary as bacteriological sensitivities became available

RESULTS

The pneumococcus was the major overall cause of meningitis salmonella species were the commonest enterobacteria

Pneumococcus accounted for 184 patients, half of whom were over 1 year of age, and many of whom were adults Throughout the first year of life (Fig 1) haemophilus influenzae and gram negative enterobacteria were

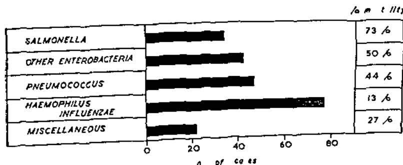


Fig 1 Organisms causing meningitis under 1 year of age in Ibadan 269 cases

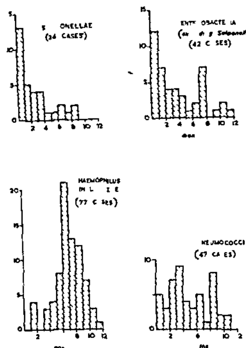


Fig 2 Age distribution of the different meningitides in Ibadan

the predominant organisms isolated Fig 2 illustrates the age distribution of the different types

Cases caused by salmonella species were similarly distributed to those caused by the other enterobacteria one third of these occurred in the first month of life during which period salmonella species was the major cause of meningitis (Fig 3) O-typing was done in 10 instances The strains identified were

Salmonella G and B 3 cases each
Salmonella A E E_1 1 case each
Salmonella typhimurium 1 case

Table 1 *Organisms comprising the miscellaneous group of meningitides in Ibadan*

	Under 1 y	Over 1 y	Total
Staph Pyogenes	10	8	18
Meningococcus	2	8	10
Haem. parainfluenza	3	2	5
Haemolytic streptococcus	0	3	3
Strep viridans	4	0	4
Strep faecalis	1	0	1
Total	20	21	41

Other enterobacteria isolated are indicated on Table 2

The miscellaneous organisms encountered are shown in Table 1 In the remaining 91 cases (44 of which were under 1 year old) no organisms were isolated but owing to the method of selection of cases this figure represents a considerable underestimate

Salmonella meningitis

The most significant finding was the high proportion of salmonella species which were resistant not only to chloramphenicol but also to tetracycline streptomycin and the sulphonamides Between 1964 and 1966 there were only two cases of salmonella meningitis showing resistance to these antibiotics at the U C H and a similar pattern was reflected amongst salmonellae causing bacteraemia These proportions have increased dramatically over the past 5 years In 1969 for instance there was only one chloramphenicol sensitive case out of the 10 salmonella meningitides and 38 out of 80 cases of salmonella bacteraemia Salmonella typhi on the other hand has re-



Fig 3 Causative organisms involved in neonatal meningitis in Ibadan 43 cases.

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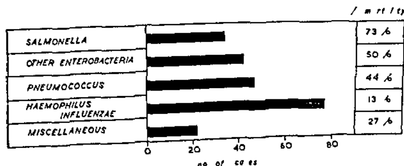


Fig 1 Organisms causing meningitis under 1 year of age in Ibadan 269 cases

Tabl. 3 *Salmonella meningitis Review of literature*

Author	Year	Source	Neonates Cases	Neonates Died	1 month + mixed Cases	1 month + mixed Died	Total Cases	Case fatality ( )
Henderson	1948	Review	49	48	98		147	96
Beene	1951	Review (+1 new)			88	72	88	84
Watson K C	1957	Durban (4 + review)	55	52	87	69	142	85 5
Reports in literature	1958–1968		2		31*		33	
Present series	1969	Ibadan	14	13	24	16	38	76

Several described as infants

have a high case fatality *per se* (5 9 23 25) in Ibadan there is in addition a resistance to antibiotics that has arisen in all groups of enterobacteria and these account for 50 % of all neonatal cases of meningitis Since there is nothing specific about the symptoms of en t robacterial infection in infants and the fa tality is high the importance of early and ener getic treatment aimed at these resistant strains cannot be over-emphasised

Antibiotic resistance of salmonella species has only been reported once previously (11) and one hopes that this lack of resistance is as real as it is apparent

SUMMARY

538 cases of bacterial meningitis admitted from January 1964 to May 1969 in all age groups at the U C H Ibadan were reviewed The proportion of enterobacterial cases of meningitis admitted to the U C H Ibadan was higher than has been reported from any other centre the maximum incidence being in the neonatal period A very large number of these were due to various salmonella species and 70 of these are currently resistant to the more commonly used antibiotics including chloramphenicol This resistance to chloram phenicol is reflected amongst all enterobacteria isolated in Ibadan and poses a considerable problem At present over 90 % of neonates with enterobacterial meningitis die and this case fatality is maintained in the older age groups in cases where resistant strains are en countered

Table 4 *Delay in appropriate therapy related to mortality*

Delay (days)	Alive	Dead	Total
0-7	8	6	14
8 14	1	4	5
15- 1	0	5	5
22	0	2	2
Total			26

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Table 2 *Enterobacterial meningitis causative organisms*

Author	E coli	Para colon	Proteus	Pseudo monas	Kleb siella	Salmo nella	B alka ligenes	Aero bacter	Unidenti fied	*Total*
Previous literature	111	27	26	13	4	*10*	*1*	*12*	*14*	*218*
Present series	9	2	3	6	7	35	—	—	40	102
Total	*120*	*29*	*29*	*19*	*11*	*45*	*1*	*12*	*54*	*320*

mained consistently sensitive to both tetracycline and chloramphenicol An analysis of 59 non-salmonella enterobacterial meningitides showed a similar trend (46% resistant), illustrated in Fig 4

Salmonella meningitis as seen in Ibadan is predominantly a disease of neonates and young infants Only 2 out of 38 patients developed the disease over the age of 1 year, at 18 months and 3 years respectively 20 were males, 16 were females, the sex being unrecorded in two There was neither seasonal variation nor evidence of *an epidemic pattern One child de-*veloped meningitis two weeks after admission for prematurity, and three patients were transferred from the Ibadan University Rural Health Training Centre, 60 miles away, but most patients were admitted directly to the U C H

The case fatality for salmonella meningitis was high in all age groups, 73 5% overall and 92 5% in the neonatal patients Five children developed subdural effusions and two of these died One child s meningitis developed secondary to an untreated otitis media, there was no obvious focus of infection in the rest

Resistance of the organism to chloramphenicol appeared to increase the likelihood of a fatal outcome, but this proved to be an artefact due to a close association with delay in treatment

DISCUSSION

Most reports of enterobacterial meningitis in infants have come from the United States or Europe (Table 2) It has not so far, been recognised as a significant problem in the tropics or developing countries Geefhuysen (1960), Pirame (1968) and Senecal (1957), reporting on large series gave relative incidences of enterobacterial meningitis compared to other types as 2%, 5%, 3% and 0% respectively Previous reports from Nigeria have shown similar figures (1, 14)

The first report of salmonella meningitis (as quoted by Mpairwe, 1968) was made by Ghon in 1908 By 1948, Henderson had collected 147 cases from the world literature Beene Hansen & Fulton reviewed 87 recent case reports in 1951 Watson collected a further 142 cases in 1958 (Table 3)

This is the largest number of new cases that have been reported from one institution, indicating that, in the period studied, salmonella meningitis is probably markedly more common in Ibadan than in other centres

The most significant prognostic factor was delay in institution of appropriate antibiotic treatment after the onset of symptoms ($p < 0.001$) This is clearly shown in Table 4

Although neonatal meningitis is known to

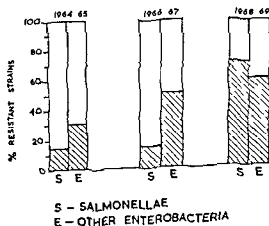


Fig 4 Increase in resistant strains of enterobacteria oveer the past 5 years

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HEMISPHERECTOMY IN EARLY HEMIPLEGIA AND INTRACTABLE EPILEPSY

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When infantile hemiplegia is associated with severe epilepsy and serious behaviour disturbances interfering with normal adjustment at home and in school hemispherectomy may be contemplated This therapy is based on the assumptions that in cases of early injury the other cerebral hemisphere in part at least assumes the functions of the affected side and that function on the intact side is disturbed by the pathological activity in the affected hemisphere the frequent convulsions and massive medication In cases of this kind hemispherectomy has been used for therapeutic purposes since the 1930s The method was first mentioned by McKenzie (11) but the chief pioneer was Krynauw (7) who reported 12 cases Since then over 400 cases have been treated by hemispherectomy (reviews by White (14) and Ignelzi & Bucy (5)) The results have been very gratifying in a large number of cases but only in a minor proportion can the effect be evaluated on the basis of observations covering many years For this reason we feel justified in presenting 6 patients treated by hemispherectomy who have been followed for 3 to 8 years

The following indications for operation have been mentioned in the literature

1) early hemiplegia 2) epilepsy refractory to ordinary doses of antiepileptic drugs 3) serious behaviour disturbances interfering with social adjustment 4) progressive impairment of intellectual performance 5) pneumoencephalographic findings indicative of unilateral involvement

As a rule a combination of all or most of these indications has been regarded as necessary

In the majority of reported cases hemiplegia has had its origin pre or perinatally or post natally in infancy or very early childhood It has not been possible to define any definite upper age limit however and the operative series comprises cases in which hemiplegia developed at school age Besides hemiplegia the main indication has invariably been frequent convulsive fits refractory to ordinary therapy The behaviour disturbances have mostly consisted of severe restlessness hyperkinesia and aggressiveness Hemiplegias due to degenerative brain disorders are contraindications to hemispherectomy On the other hand increasing dementia caused by anoxic injuries during severe seizures and excessive anticonvulsant medication are important indications It is considered important that the pneumoencephalographic findings are largely limited to one hemisphere They consist of dilatation of the lateral ventricle on the affected side and a shift of the midline towards this side (7) The electroencephalogram does not have the same prognostic value Although patients with

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unilateral changes seem to have the best prognosis (13) bilateral changes of the background activity and bilateral spikes and waves do not preclude good results (7)

The surgical technique varies to some degree Part of the temporal lobe is left by some surgeons while the majority today excise the entire lobe The incision through the region of the basal ganglia is now perhaps most often made between the nucleus caudatus, most of which is excised, and the thalamus, which is left for the most part (9) The operative mortality has ranged from 5 to 10%

A good or satisfactory result has been reported in the vast majority of cases As a rule the hemiplegia is transiently impaired Initially a flaccid paresis results but usually the end result is reduced spasticity and a wider range of motion than preoperatively The drawback is a loss of thumb capacity for opposition and circumduction finger capacity for piano movements and hand capacity for supination as well as impairment of sensation (8) The epileptic attacks are completely abolished in two thirds of the patients and alleviated in at least half of the remainder Largely similar results have been noted in regard to the behaviour disturbances Neither behaviour nor epilepsy have been adversely affected by the operation (4)

Postoperative improvement in their IQ scores has been reported for many patients (10) The improvement is probably due to increased mental efficiency and better concentration A decrease in the IQ scores has been noted in about 10% of cases

As an additional handicap homonymous hemianopsia develops in all cases in which it was not already present

On the other hand the most dreaded kind of additional disability, i e aphasia resulting from excision of the dominant hemisphere has not proved important (1) Transient aphasia has been reported in a minor proportion of the cases mainly in those in which hemiplegia developed after the patient started talking but equally often in association with right sided as with left sided hemispherectomy In some patients the development of speech was accelerated after the operation

During the last few years the enthusiasm for hemispherectomy has decreased owing to the late complications reported in some operative series (2, 3, 12) These complications originate in a cyst formed at the site of the excised hemisphere The cyst may be separated from the remainder of the subarachnoid space, with local expansion or hydrocephalus resulting or there may develop in its wall a granulating often bleeding membrane resembling that seen in subdural haematoma The bleeding causes haemosiderosis and irritation of the ependyma and meninges, leading to the formation of adhesions and hydrocephalus These complications may occur as much as 6 to 12 years postoperatively, and it is believed that they may occur in up to one fourth of the cases

MATERIAL

The present series consists of 3 girls and 3 boys aged 4 to 17 years at operation (see Table 1) In 4 cases the cerebral lesion and the spastic hemiplegia were probably due to birth injury In 2 of these patients the condition deteriorated markedly in connection with a protracted epileptic fit at the age of 3 and 5 years respectively One girl had an intracerebral haemorrhage at the age of 1 year probably caused by rupture of an aneurysm in the a communicans on the right In one case the etiology is unknown but it seems possible that encephalitis was involved The left hemisphere was involved in 4 cases the right hemisphere in 2 Convulsive attacks were the main indication for operation in all cases In spite of anticonvulsant medication for many years the fits had increased in frequency until they occurred many times a day or even many times within an hour Various types of partial seizures were represented as a rule with secondary generalization

Five of the 6 patients showed behaviour disturbances In 3 cases these were so serious that school attendance and continued care at home had become impossible (hyperkinesia aggressiveness destructibility) All of these 5 patients showed steady impairment of intellect In 3 cases the preoperative IQ had dropped to the level of moderate to severe mental retardation (Fig 1) One boy was too restless to take a psychological test before the operation

The pneumoencephalographic finding was pathological in all cases Four showed the picture which is optimal from the standpoint of the result of hemispherectomy i e dilatation of one lateral ven

Table 1 *Six cases of hemispherectomy preoperative data*

Case no	Sex	Age at operation (yrs)	Hemiplegia: Age at onset	Hemiplegia: Cause	Epilepsy type and frequency of attacks	Behaviour	Intelligence: Age (yrs)	Intelligence: IQ	Pneumo-encephalography
1	♀	10	5 yrs	? Encephalitis	Partial and generalized motor many daily	Hyperkinetic aggressive	6 9	106 74	Dilated right ventricle *shift to right*
2	♂	8	1 yr	Rupture of aneurysm	Partial motor 2–3 daily	Hyperkinetic noisy uncontroll	2 5 8	105 90 57	Dilated right ventricle shift to right
3	♂	17	First months	Birth injury	Partial motor and aphasic up to 10 *daily*	Not abnormal	13	49	Dilated left ventricle shift to left
4	♀	11	First months deteriorated at 5 yrs	Birth injury & injury during epileptic fit	Partial and generalized *motor* many daily	Restless aggressive	7 11 severely demented	49	Especially left ventricle but also right and third ventricles and basal cisterns dilated
5	♂	4	First months deteriorated at 3 yrs	Birth injury & injury during epileptic fit	Partial and generalized temporal absences up to 20 per hour	Hyperkinetic, aggressive destructive	Not tested (severe restlessness)		Dilated left ventricle
6	♂	13	First months	Birth injury	Partial and generalized many daily	Restless aggressive	8 10 13	78 72 30	Severely dilated left ventricle right and third ventricle and basal cisterns somewhat dilated

tricle without enlargement of the other ventricle or of the cisterns Three of these patients showed a lateral shift of the midline of over 5 mm towards the affected side (Fig 2) In the other two cases there was also slight dilatation of the other lateral ventricle the third ventricle and the basal cisterns (Fig 3)

In all patients but *one* the electroencephalogram showed a disturbance of the background activity of both hemispheres and spike–wave paroxysms which

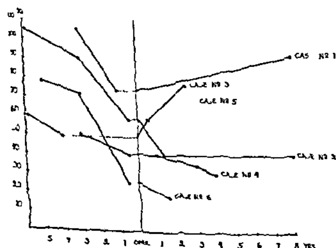


Fig 1 The intelligence quotient (IQ) before and after hemispherectomy

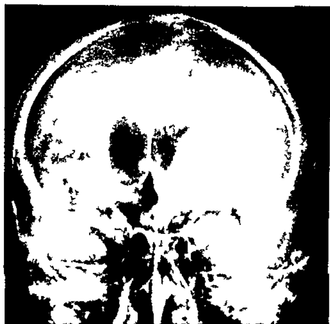


Fig 2 Case 1 PEG findings before hemispherectomy enlarged right ventricle (the distance from septum pellucidum to nucleus caudatus 17 mm at the right and 12 mm at the left) Shift to the right of the middle structures (7 mm) Other ventricles and cisterns of normal size

often were generalized Asymmetry was seen in all patients however and in one case the spikes and spike wave complexes were unilateral only

TREATMENT

The same operative technique was employed in all cases It has previously been described (6) and does not deviate from the method described in the literature

In one case (No 2) a postoperative complication occurred in the form of bleeding from a venous sinus which caused a haematoma entirely filling the operative cavity After recraniotomy and excision of the haematoma this patient also recovered although more slowly than the remainder

Postoperatively all patients initially showed massive contralateral hemiplegia In this stage none of them had any epileptic fits During the immediate postoperative stage all the patients showed aggressiveness towards the nursing staff Five children were transferred at an early stage to a pediatric hospital with good possibilities for rehabilitation where the anticonvulsant therapy was successively modified and reduced Patient No 6 returned home after a short stay at an institution for retarded children In this case rehabilitation was inadequate

FOLLOW-UP INVESTIGATION

The interval between hemispherectomy and follow-up investigation ranged from 3 to 8 years The patients ages at follow up ranged from 8 to 25 years (Table 2) In addition to





Fig 3 Case 4 PEG findings before the operation Both ventricles dilated the left more (Evans index 0 40 at the left and 0 32 at the right) Shift to the left of the middle structures (3 mm) The third ventricle is dilated (horizontal diameter 11 mm) Basal cisterns dilated

Table 2. Six cases of hemispherectomy: postoperative data

Case No.	Age at follow up exam (yrs.)	Duration of follow up (yrs.)	Side of oper.	Complications	Function of arm and leg	Epileptic fits	Intelligence, school work or care	Behaviour	Disturbance of vision	Dysphasia
1	18	8	Right	Osteitis at site of oper.	Arm not used, walks with slight limp	Stopped (5 yrs later some autonomic attacks)	Improved IQ 91 finished primary school, attends peoples high school	Cheerful euphoric passive concentrates well	Homonymous hemianopia	None
2	17	8	Right	Early post oper. intracranial bleeding	Arm not used, slight limp	Stopped	Slight deterioration IQ 40 centre for day care	Improved lively unconcentrated	Only half of right field intact	None
3	5	8	Left	—	Arm not used, slight limp	Stopped	Improved IQ 77 shop-assistant	Normal (as before operation)	Homonymous hemianopia	Same degree and type as before operation
4	15	4	Left	—	Arm not used, slight limp	Less severe, fewer attacks	Slight deterioration IQ 30 institutional care	Improved cheerful passive	Homonymous hemianopia	None
5	8	3	Left	Transient respiratory & cardiac arrest 3 months after oper.	Arm not used, does not walk after complic.	Stopped ? small temporal absences 3 yrs after oper.	IQ 68 class for subnormal CP children	Improved passive cheerful concentrates	Only half of right field intact	None
6	16	3	Left	Periodical headache	Arm not used, walks with difficulty	Only transient alleviation	Further deterioration IQ 0 cared for at home	Improved passive apathetic	Homonymous hemianopia	Difficult to assess

the above mentioned severe complication in the acute stage in case 2, a severe complication also occurred in another patient (No. 5) 3 months after operation. The primary postoperative result was very good in this case, but after 3 months this boy developed pressure symptoms which within 3 weeks led to respiratory and cardiac arrest, necessitating protracted respirator treatment. No drainage was applied. It may be assumed that the condition was due to blocking of the outflow from the cyst at the site of the excised hemisphere, resulting in expansion. In these 2 cases the results were impaired by the complications. In addition one patient (No. 1) showed protracted osteitis in the operative area, and another patient (No. 6) experienced frequent episodes of headache during the first 2 years.

After hemispherectomy 3 patients started walking within a few weeks, while 2 patients with a low IQ and that girl who had a postoperative haemorrhage did not walk until 1 to

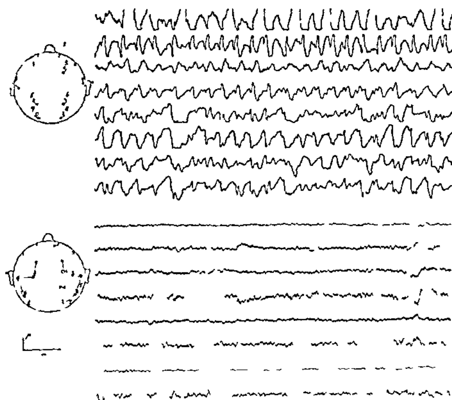


Fig 4 Case 1 EEG registered before the operation and 8 years after the operation (the most pathological segments have been chosen)

2 years postoperatively The postoperatively occurring severe complication caused worsening of the handicap in case No 5 After this he has not been able to walk without help None of the 6 patients is able to use the hemiplegic hand which is kept closed with the arm flexed in adduction In at least 3 cases the sense and stereognostic ability on the hemiplegic side is poorer than before operation Sensation in all modalities is diminished, and one patient sustains frostbite in the hand without noticing

The epileptic fits were immediately abolished in 4 cases Later one of these patients has had brief partial seizures with motor and autonomous symptoms during a short period and another has experienced temporary absences of short duration In one patient the fits were alleviated while in another (No 6) only transient improvement of short duration was noted and the epilepsy is now as severe as before operation The electroencephalogram in all cases showed markedly reduced activity on the side of the operation while the background activity on the contralateral side was normal in 3 cases and slightly pathological in 3 Spike-wave paroxysms occur in those 2 cases in which attacks have persisted The other 4 patients show occasional sharp transients only (Fig 4)

Two patients show significant improvement in their IQ scores while 3 show a decrease (Fig 1) This group consists of those 2 patients in whom seizures have persisted and that girl who had severe postoperative bleeding One patient (No 5) could not be tested until after the hemispherectomy In spite of the above mentioned severe complication involving respiratory failure and further disablement, he has now an IQ of about 60–70 The behaviour of our patients has radically changed for the better in that restlessness and aggressiveness have disappeared They have become cheerful and calm and better able to concentrate on their tasks than before Excessive passiveness is now the greatest problem One patient showed no disturbances of behaviour before operation nor after and one patient is still restless though not to the same extent unrestrained and aggressive as preoperatively

Four patients show homonymous hemianopsia but otherwise their vision is good In 2 patients one eye is almost blind in one

owing to the primary disorder in the other (No 5) as a result of complicating high intracranial pressure Thus these 2 patients can only use half the visual field of one eye A girl aged 18 who shows the best result of hemispherectomy generally speaking states that the hemianopsia and disturbance of sensation in the hand are now her chief complaints

Speech has not been impaired in any of our patients although 4 of them were subjected to left sided hemispherectomy In that patient who has the lowest IQ it is difficult to evaluate the result in this regard however

Two of our patients were able to attend school after the hemispherectomy and have passed primary school One of them then received occupational training and is working as a shop assistant The other one is aiming at further studies at an adult continuation school after 1 year s attendance at evening courses One child attends a class for subnormal CP children while 3 patients are moderately or severely mentally retarded Two of these are cared for at home and one at an institution for mentally retarded where she works at the loom

CONCLUSION

We thus have 2 satisfactory cases (Nos 1 and 3) and one in which the operation was of no benefit (No 6) In 3 cases the result is neither altogether satisfactory nor poor Two of these patients have a low IQ but one of them (No 2) has been entirely relieved from epileptic fits and the other (No 4) from aggressiveness and restlessness although epilepsy persists In case No 5 the primary result was good but additional disablement was caused by a late complication

Considering that those 2 of our 6 patients in whom pneumoencephalography showed signs of more extensive damage had the poorest prognosis (these two did not have complications) we would not recommend hemispherectomy if the pneumoencephalographic findings are not strictly unilateral Severe mental retardation is also a contraindication as the rehabilitation of these patients after the operation is very difficult The occurrence of complications many years after operation reported by many authors since 1966 is also an argument for cautiousness

SUMMARY

Six children and young persons are describ d in whom hemispherectomy was performed because of intractable epilepsy and in 5 severe behaviour disturbance All of them had spastic hemiplegia and progressive impairment of the intellectual function before the operation The follow up period was 3 to 8 years In two cases the results were satisfactory in 3 cases only partly so Two of these had severe complications one immediately after the operation and one some months later the complications probably impaired the results One boy did not benefit from the operation

Addendum Recently Wilson has published an extensive report on the early and late results of hemispherectomy Wilson P J E Cerebral hemispherectomy for infantile hemiplegia A report of 50 cases *Brain 73* 147 1970

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ORAL D PENICILLAMINE AND INTRAMUSCULAR BAL+EDTA IN THE TREATMENT OF LEAD ACCUMULATION

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While symptomatic lead poisoning continues to be a problem the presence of abnormally high concentrations of lead in the tissues of asymptomatic children may have subtle but disastrous effects Among others Moncrieff et al (9) and Gibson et al (5) demonstrated the presence of lead accumulation in many retardates Although this work cannot be fully assessed at the present time it raises the possibility that unsuspected lead accumulation may be a significant etiological factor in the retardation of otherwise asymptomatic children. In addition many asymptomatic children with elevated lead levels may go on to develop overt plumbism These factors make deleading therapy mandatory

Several substances have been used in treatment While the combination of BAL and EDTA has been shown to be an efficient deleading regimen it must be given parenterally and there are a number of side reactions (1 8) It is therefore recommended in the literature only for severe acute lead encephalopathy (1 3) D-penicillamine is an effective lead chelator when given orally and is usually well tolerated (1 6 10)

In the present study the effects of these two therapies were compared in 3 children with demonstrated lead accumulation D penicillamine appeared to delead as effectively as BAL and EDTA

The patients were studied in the Yale University Children s Clinical Research Center supported by Public Health Service Grant FR-00125 also partially supported by the Publi Health Service (T1 AM 5351).

CASE REPORTS

Case 1 J H (58 32 13) a 34-month old Negro girl had been found eating paint from a paint can 2 months prior to admission She had also been noted to peel and ingest cracked paint from the walls A blood lead determination 1 month before admission was 80 μg per 100 ml

At the time of admission she was found to be an alert cheerful and healthy looking girl There were no ophthalmological or neurological abnormalities and the examination was unremarkable Her height was 92 cm (50th percentile) and weight 13 8 kg (40th percentile) Psychological testing determined that she was functioning at the average mental level for her age

An EEG was normal Radiological survey showed dense zones of subepiphyseal sclerosis in the distal femora proximal tibiae and distal long bones of the wrists Abdominal radiograms revealed a few areas of density in the sigmoid

There was minimal proteinuria glucosuria and generalized aminoaciduria the patterns of urine amino acids varied throughout the course of treatment Additional laboratory data are given in Table 1

Case 2 M O (63 05 71) a 25 month-old Puerto Rican girl undergoing treatment for ascariasis was found by a visiting nurse to be eating paint chips from the walls of her home The blood lead was 100 μg per 100 ml and the patient was admitted for study

Physical examination revealed a girl who appeared to be anemic Her height was 88 cm (60th percentile) and her weight 12 7 kg (0th percentile) The rest of the physical examination including neurological evaluation was entirely negative Her developmental evaluation was consistent with her age

An EEG was within normal limits Radiological survey of the epiphyses showed very dense wide subepiphyseal bands of sclerosis No radiopaque densities were seen in the bowel

Table 1 *Laboratory data*

	Patient 1								Patient 2							
	Adm	I	II	III	IV	VI	VII	VIII	Adm	I	II	III	IV	VI	VII	VI
Blood																
Hemoglobin (g/100 ml)	11 9	11 9	11 1	11 5	11 6	11 9	10 6	10 5	10 7	10 9	11 5	9 7	10 0	9 0	9 1	7
Reticulocytes ( )		0 9	1 2	2 4	3									4 0		
WBC ($\times 10^3$/mm^3)	6 1			6 6	8 5	5 6	5 7	4 8	11 2	6 6	10 1		7 5	8 0	8 2	5
Neutrophils ( )	*46*				*71*	*15*	*40*	*59*	*30*	*33*	*17*		*43*	*26*	*53*	*34*
Lymphocytes ( )	43				27	74	50	35	59	57	55		41	56	35	50
Eosinophils ( )						5	4	4	10	7	20		4	14	5	12
Bilirubin (mg/100 ml)	0 6							0 5		0 4			0 3			0
SGOT (K U)	55							107		27			37			10
Thymol floc	0							0		0			0			0
Cephalin floc	0,0							0,0		0 0			0 0			0 3
Thymol turbidity								1		2			2			2
Alk Phos (B U)	13							4		34			15			7
CSF																
Lead (μg/100 ml)											60		10			
WBC	0										1		1			0
Protein (mg/100 ml)	16										32		20			20

Aminoaciduria of no specific pattern was noted throughout hospitalization Additional laboratory data are given in Table 1

Case 3 D C (64 92 26) a 9 year old Puerto Rican boy was one of a large family of children who had been treated for plumbism A visiting nurse referred him to the clinic where a blood level of 160 μg per 100 ml was found and he was admitted for study

He was a short shy pale boy The height of 100 cm and the weight of 10 9 kg were both below the third percentile No ocular abnormalities were found and except for bilateral positive Babinski signs the remainder of the physical examination was normal The developmental evaluation placed him in the educable range

An EEG demonstrated findings suggestive of some disorganization of the normal faster rhythms during the waking state The findings were diffuse and compatible with some diffuse cerebral dysfunction

X rays of the hands revealed some epiphyseal sclerosis and growth lines in the distal forearm while the knees manifested multiple growth lines and accentuation of the subepiphyseal sclerosis consistent with heavy metal ingestion The bone age was at the 6 to 7 year level

Minimal proteinuria glucosuria and aminoaciduria of a non specific type were observed throughout the hospitalization Additional laboratory data are given in Table 1

The child s stools contained Trichuris trichiura and hookworm ova for which he was treated after completion of the study

METHODS

The patients were selected according to the following three criteria treatment for plumbism for the first time a blood level of at least 70 μg per 100 ml and no necessity for emergency treatment They were studied in the Yale Children s General Clinical Research Center After an initial period of 3-6 days during which the child became adjusted to the hospital environment and the diet was prepared there followed 8 three day periods

I and II No therapy
III and IV D penicillamine 100 mg kg/day in divided doses given every 6 hours orally
V and VI No therapy
VII and VIII BAL 24 mg kg day in divided doses given every 4 hours and EDTA 75 mg kg/day mixed with procaine in divided doses every 4 hours given intramuscularly

Each patient received three different daily menus alternating for each 3 day period Duplicates of the food and fluids offered to each patient during the first and last period were assayed for lead content as were the unconsumed portions of each meal The diets for periods I and VIII and the left overs for all the periods were each homogenized and portions were frozen until analysed The difference between the average of the lead consumed during the first and eighth periods and the lead content of the unconsumed food was taken to represent the lead intake

All samples were collected and stored in acid washed bottles Twenty four hour urine samples were collected on each day of the study with the exception of period V The daily urines were then combined into a single sample for each 3 day study period and an aliquot was frozen until used for analysis

Stools were collected for 72 hour periods using alternating charcoal and carmine red markers The collections began with the first appearance of the marker and ended with the first evidence of the next

Patient 3

A'n	I	II	III	IV	VI	VII	VIII
10 0	9 9	9 9	10 3	9 4	10 2	9 6	10 00
	3 6	3 4		1 8	1 5	2 0	
1^0		9 5	1 1	13 3	7 7	8 7	11 6
5		51	60	53	46	58	61
8		79	21	36	27	31	28
11		13	12	7	18	7	6
0 3				0 5			0 2
1				28			151
0				0			0
0 0				0/1 +			0 0
3				2			1
				6			7
10				150			
7							
							1
							0

marker All stools were homogenized and an aliquot was kept frozen

Lead determinations on the blood and cerebrospinal fluid were performed by the Laboratory Division of the Connecticut State Department of Health on ashed aliquots by the dithizone extraction method as modified by Woessner & Cholak (11) and Cholak et al (2) Urine stool and diet specimens were examined for lead by the same method at the Occupational Medicine Service Industrial Hygiene Laboratories of the Massachusetts Institute of Technology

On the last day of each period blood was obtained for a complete blood count for blood lead and for determination of electrolytes protein calcium magnesium iron phosphorus and ceruloplasmin concentrations and for liver function tests Urine was tested for creatinine protein and glucose excretion and for amino acid and sugar chromatography Spinal fluid was obtained when blood lead levels were high

RESULTS

Balance studies

These are shown in Fig 1 together with the corresponding blood lead concentrations There was a striking decrease in the blood lead levels to normal range after 6 days of either treatment All of them showed a rebound however on the sixth day after discontinuation of the first medication D penicillamine The increase in lead excretion in the urine during the periods of treatment was striking and there was a direct relationship between the blood lead concentration before treatment and the amount of lead excreted In all patients the stool content of lead was higher than that ingested in the diet with the exception of the first two control periods on Case 1

The quantities of lead excreted during the treatment periods are shown in Table 2

Other studies

Serum electrolytes protein calcium phosphorus iron and magnesium levels as well as ceruloplasmin concentrations remained constant during the 8 periods

Several abnormalities of the blood were noted (Table 1) There was a mild reticulocytosis in all 3 patients An eosinophilia was present in all Neutropenia was noted once in Case 1 during period VI

Liver function tests on all patients showed an increase of SGOT to abnormal values after BAL + EDTA treatment In 2 of the 3 patients the cephalin flocculation test became strongly positive at 48 hours during the BAL + EDTA treatment a positive result was also obtained in Case 3 during penicillamine treatment The alkaline phosphatase in Case 2 which was elevated before treatment showed a decrease to the normal range

No significant changes were observed in urinary amino acids or in the results of routine urinalysis

DISCUSSION

D penicillamine appeared to be as effective a deleading agent as BAL + EDTA in these studies A more definitive comparison cannot be made on the basis of our data however since D-penicillamine was the first drug administered and therefore may be presumed to have interacted with a larger absolute and proportional amount of "labile lead More rigorous comparison would require alternation of the sequence of administration of the drugs and greater number of cases The adequacy of D penicillamine as a plumburetic agent is established however from the present as well as previous studies (1 6 10)

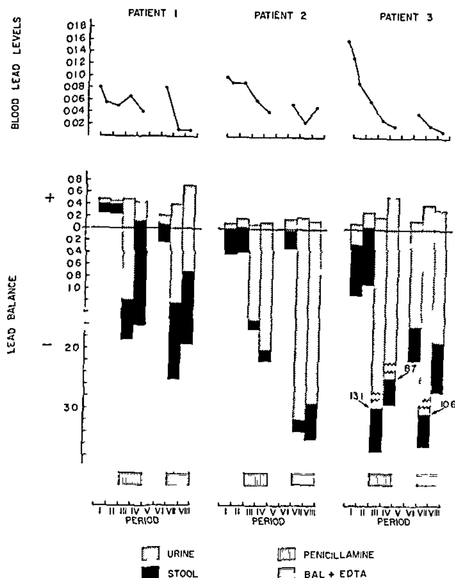


Fig 1 The effects of chelating agents upon lead balance Blood lead levels are expressed in mg/100 ml During the indicated periods the top of the bar indicated the amount ingested (mg/period) the urine and stool lead content are then plotted negatively so that the total height of the bar represents the lead excretion in mg/period

It is pertinent that the administration of D-penicillamine was not especially regulated with respect to food ingestion It is quite possible that its efficiency would have been greater were it given on an empty stomach as is customary in treating patients with Wilson's disease

The dosage of D penicillamine which we employed was higher than that implied by Gibbs & Walshe (4) and by Chisolm (1) to be a safe maximum about 40 mg/kg/day According to these authors the minimum time preceding the development of signs of pyridoxine deficiency was 2 months of uninterrupted use It should be stressed however, that a D penicillamine dose level as high as 100 mg/kg/day, was well tolerated in our 3 patients Jaffe et al (7) found that it took 2 weeks of uninterrupted administration of about the same dose as that administered by Gibbs & Walshe (4) to produce biochemical but not clinical evidence of pyridoxine deficiency

Table 2 *Cumulative negative lead balance during chelation therapy*

	D Penicillamine	BAL + EDTA
Case 1	3 47 mg	4 42 mg
Case 2	3 90 mg	6 92 mg
Case 3	22 45 mg	13 90 mg

The rebound in blood lead concentration

noted 6 days after discontinuing treatment is not unusual in the use of chelation therapy in general Repeated courses of treatment until the rebound concentration is within acceptable limits is a standard procedure

While D-penicillamine has been given parenterally in some studies (10) it is its ability to function effectively when administered orally that makes it the preferable drug for deleading the asymptomatic child The pain restraint and bewilderment accompanying injections can be avoided This is an advantage especially in the management of children of the age and socioeconomic background most likely to have accumulated lead It must be remembered however that chelators are transporters and that their affinities for their ligands are relative not absolute "Dietary" lead thus could be transported to the tissues by orally administered D-penicillamine so that the latter should not be used unless it is certain that no lead remains in the intestinal tract and that the child is no longer exposed to the source

Although potentially serious hematological difficulties have been encountered early in the course of penicillamine treatment (8) these have been reversible and would not appear to contraindicate the use of the drug In our patients neutropenia was noted only once and this was moderate and transient The mild reticulocytosis noted in our patients was noted on admission in one and may in the others have been due to blood sampling or to the lead accumulation itself The eosinophilia noted in 2 patients was present at the time of admission

It is of interest that the only sign of liver toxicity an elevation in transaminase occurred during BAL EDTA administration and that no urinary abnormalities were noted during either regimen

We conclude that short term oral administration of D penicillamine is a convenient and effective means of removing lead from the body provided that (i) the patient is well enough to be cooperative (ii) has no lead in his gut and (iii) is no longer exposed to ingestible lead containing material Furthermore this use of penicillamine would appear to offer no more and more likely, less of a potential hazard than BAL+EDTA or than EDTA alone It is to be stressed that both regimens are potentially toxic and require careful monitoring

SUMMARY

Oral D-penicillamine and intramuscular BAL+EDTA have been used in the treatment of lead accumulation in three children with blood levels of at least 70 μg per 100 ml Lead balance studies were done during control periods and while the children were given either D penicillamine orally or BAL plus EDTA intramuscularly There was a marked cumulative negative lead balance and the blood lead levels decreased to normal after 6 days with either treatment The results indicate that D penicillamine given to three children orally in doses as high as 100 mg/kg/day is an effective and non toxic lead chelator for short term use

ACKNOWLEDGEMENT

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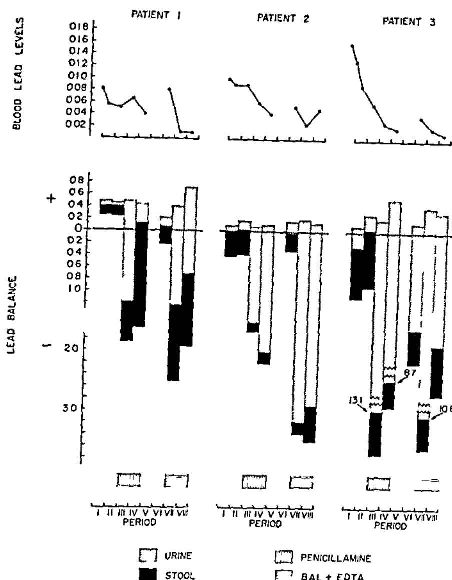


Fig 1 The effects of chelating agents upon lead balance Blood lead levels are expressed in mg/100 ml During the indicated periods the top of the bar indicated the amount ingested (mg/period) the urine and stool lead content are then plotted negatively so that the total height of the bar represents the lead excretion in mg/period

It is pertinent that the administration of D penicillamine was not especially regulated with respect to food ingestion It is quite possible that its efficiency would have been greater were it given on an empty stomach as is customary in treating patients with Wilson s disease

The dosage of D penicillamine which we employed was higher than that implied by Gibbs & Walshe (4) and by Chisolm (1) to be a safe maximum about 40 mg/kg/day According to these authors the minimum time preceding the development of signs of pyridoxine deficiency was 2 months of uninterrupted use It should be stressed however that a D penicillamine dose level as high as 100 mg/kg/day, was well tolerated in our 3 patients Jaffe et al (7) found that it took 2 weeks of uninterrupted administration of about the same dose as that administered by Gibbs & Walshe (4) to produce biochemical but not clinical evidence of pyridoxine deficiency

The rebound in blood lead concentration

Table 2 *Cumulative negative lead balance during chelation therapy*

	D Penicillamine	BAL + EDTA
Case 1	3 47 mg	4 42 mg
Case 2	3 90 mg	6 92 mg
Case 3	22 45 mg	13 90 mg

noted 6 days after discontinuing treatment is not unusual in the use of chelation therapy in general Repeated courses of treatment until the rebound concentration is within acceptable limits is a standard procedure

While D-penicillamine has been given parenterally in some studies (10) it is its ability to function effectively when administered orally that makes it the preferable drug for deleading the asymptomatic child The pain restraint and bewilderment accompanying injections can be avoided This is an advantage especially in the management of children of the age and socioeconomic background most likely to have accumulated lead It must be remembered however that chelators are transporters and that their affinities for their ligands are relative not absolute "Dietary lead thus could be transported to the tissues by orally administered D-penicillamine so that the latter should not be used unless it is certain that no lead remains in the intestinal tract and that the child is no longer exposed to the source

Although potentially serious hematological difficulties have been encountered early in the course of penicillamine treatment (8) these have been reversible and would not appear to contraindicate the use of the drug In our patients neutropenia was noted only once and this was moderate and transient. The mild reticulocytosis noted in our patients was noted on admission in one and may in the others have been due to blood sampling or to the lead accumulation itself The eosinophilia noted in 2 patients was present at the time of admission

It is of interest that the only sign of liver toxicity an elevation in transaminase occurred during BAL + EDTA administration and that no urinary abnormalities were noted during either regimen

We conclude that short term oral administration of D penicillamine is a convenient and effective means of removing lead from the body provided that (i) the patient is well enough to be cooperative (ii) has no lead in his gut and (iii) is no longer exposed to ingestible lead containing material Furthermore this use of penicillamine would appear to offer no more, and more likely less of a potential hazard than BAL+EDTA or than EDTA alone It is to be stressed that both regimens are potentially toxic and require careful monitoring

SUMMARY

Oral D penicillamine and intramuscular BAL+EDTA have been used in the treatment of lead accumulation in three children with blood levels of at least 70 μg per 100 ml Lead balance studies were done during control periods and while the children were given either D penicillamine orally or BAL plus EDTA intramuscularly There was a marked cumulative negative lead balance and the blood lead levels decreased to normal after 6 days with either treatment The results indicate that D penicillamine given to three children orally in doses as high as 100 mg/kg/day is an effective and non toxic lead chelator for short term use

ACKNOWLEDGEMENT

We wish to acknowledge the assistance of Dr Rudolph K. Waldman and Mr Mario Fresina for their help in carrying out some of the lead determinations

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Greece

Key words Lead poisoning plumbism penicillamine BAL EDTA chelators

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INTERMITTENT MUSCULAR WEAKNESS EXTRASYSTOLES AND MULTIPLE DEVELOPMENTAL ANOMALIES

A New Syndrome?

ELLEN DAMGAARD ANDERSEN PETER A KRASILNIKOFF and HANS OVERVAD

From Medical Department B Department of Paediatrics Department of Odontology University Hospital of Copenhagen Copenhagen Denmark

The co-existence of multiple anomalies in a patient has always attracted physicians atten tion particularly if the patient s appearance deviates grossly from normal Usually in such a case an attempt is made to find a single pathogenic factor In a few cases the diag nosis suggests itself to the physician already after his first glance at the patient Examples of this kind include the syndromes of Marfan and Down Most cases however are much more difficult to clear up and in a number of instances no definite diagnosis can be established

In the following paragraphs the case history of an 8 year-old boy will be presented He has several signs and symptoms suggesting a syndrome However on searching the litera ture we have not been able to find a descrip tion which fits our case It is hoped that this paper might prompt other physicians to re port on similar patients

CASE REPORT

The patient was born in June 1962 His mother had previously had four abortions but has given birth to a normal girl in 1966 His father suffers from psoriasis Otherwise both parents are healthy His mother s sister has primary amenorrhoea Apart from that, the family history has revealed nothing of im portance

The pregnancy was complicated by a minor vaginal bleeding in the second month for which the mother was treated with hormone injections of unknown type The boy was born at term and delivery was normal His birthweight was 3 000 g Several developmental anomalies were noticed soon after birth a large soft cranium with incomplete mineralisation of the frontal and parietal bones a defect of both the soft and the osseous palate a single transverse palmar crease of both hands and cryptorchidism Three months later extrasystole was found at a local hospital but no other cardiac signs or symptoms were present Control at irregular intervals during the following years showed an unchanged frequency of extrasystoles Physical development seemed to proceed normally the boy was able to sit without support at the age of 7 months and he could walk 7 months later Since an unsuccessful attempt at surgical repair of the palatal defect in 1964 the boy has been wearing a palatal plate

In June 1969 the boy now aged 7 was admitted to a local hospital after an episode of muscular weak ness of 3 / hours duration during which he had been unable to raise his arms and legs The most prominent finding was coupled ventricular extra systoles because of which he was digitalized He was discharged on a regimen of digoxin 0 125 mg per day This treatment did not seem to have any effect on the number of extrasystoles

One month later he was readmitted after a short lasting syncope and a dizzy spell followed by muscular weakness No cyanosis dyspnoea convulsions or nystagmus were noted On June 15th 1969 the pa tient was transferred to the Paediatric Department Rigshospital Copenhagen for further investigation

On admission he measured 110 cm which is 18 cm below normal for his age His bodyweight was 16 1 kg this is 2 kg below normal for his height His appearance was peculiar with scaphocephalic skull

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Fig 1 Eight year old boy with low set ears hypertelorism broad root of the nose mandibular hypoplasia and scaphocephalic cranium

hypertelorism low set ears broad nose hypoplasia of the mandible thin hair and slight bilateral ptosis (Fig 1) Body proportions were normal His fingers were short and pointed with an inward bending of the fifth fingers (Fig 2) and his fifth toes were overlaid His testicles could not be palpated No cardiovascular abnormalities could be demonstrated apart from an irregular heart rate resulting from extrasystoles There was no praecordial thrill and no significant murmurs The second sound over the pulmonary area was normal A phonocardiogram was also normal An ordinary neurological examination disclosed weak patellar reflexes on both sides but normal muscular strength and tone There was no sign of dyscoordination and the cutaneous sensibility was *normal*

An intraoral examination showed normal mucous membranes In the central part of the palate a defect measuring $1 \times {}^{1}/_{2} \times 2$ cm was found while the posterior part of the hard palate and the soft palate was characterized by cicatricial changes and by lack of uvula (Fig 3)

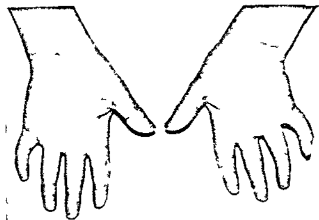


Fig 2 Hands with short and pointed *fingers inward* bending of the fifth fingers and transverse furrow in the palms

The following teeth were present (nomenclature according to Haderup (3))[1]

	05	04	03	02	01	+	01	02	03	04	05
6	05	04	03	02	01	−	01	02	03	04	05

All teeth were of normal shape and colour There was a cross bite on +04 −04 +05 −05 04+ 04− 05+ and 05− and 02+ was inverted lingually There was an open bite in the front region measuring 4 mm Radiological examination showed aplasia of 7− 5− 4− −4 −5 −7 1− and −1 The stage of development of the tooth germs was late

[1] According to the Haderup system of dental designation + signifies the maxilla − the mandible If *the symbol is placed to the right of the* figure the right side is indicated and vice versa 0 before the figure indicates a primary tooth



Fig 3 Central defect of the hard palate surrounded by cicatricial changes after surgical intervention

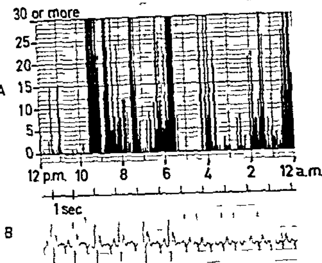


Fig 4 (A) 12 hour histogram showing the number of ectopic beats per minute Read from right to left (B) Characteristic rhythm strip demonstrating a change from bigemini to normal sinus rhythm Bipolar chest lead ectopic beats indicated by dots

The laboratory tests referred to below have been divided into different groups in order to evaluate the following diagnostic possibilities

A Chromosomal anomaly

cultures of both leucocytes from the blood and cells from a skin biopsy showed a chromosomal pattern as found in the normal male

B Neuromuscular disease

electromyography of the right biceps and left anterior tibial muscles and of the hypothenar muscles after repetitive stimulation of n ulnaris disclosed no signs of neuromuscular disease The blood concentrations of the following intracellular enzymes were all normal glutamate pyruvate transaminase (0 5 units/liter) lactic acid dehydrogenase (LDH) (20 u/l) with a normal distribution of LDH isoenzymes aldolase (?? u/l) and alkaline phosphatase (78 u/l) The creatin phosphokinase was slightly but not constantly elevated (141–45 u/l) The urinary excretion of creatin and creatinin was also within the normal range for his age 50 mg and 390 mg respectively Serum potassium was 4 7 mEq/l These investigations were carried out when the patient was free from symptoms

C Endocrine disease

the plasma level of protein bound iodine (5 1 μg/100 ml) the uptake of radioactive tri iodothyronine by the patient's serum and the urinary excretion of 17 keto-steroids (0 4 mg day) and 17 ketogenic steroids (3 7 mg/day) were all normal The administration of metyrapon resulted in an increased excretion of 17 ketogenic steroid in the urine (12 5 mg/day) and a significant fall of the plasma cortisol (1 5 μg/100 ml) while compound S increased (17 7 μg 100 ml) X ray of the skull (including sella turcica) showed a relative underdevelopment of the facial bones and several ossa Wormiana but was otherwise normal X ray of the whole skeleton showed no abnormalities the centres of ossification and the epiphyseal lines were normal

D Other investigations

Haemoglobin 14 2 g 100 ml Leucocyte count 4 300/mm Prothrombine time 107% Serum sodium 146 mEq/l Serum calcium 10 4 mg/100 ml Serum phosphorous 5 4 mg/100 ml Blood urea 14 mg/100 ml Serum creatinine 0 6 mg 100 ml Urine microscopy normal IgA 0 93 g/l IgG 10 9 g/l IgM 0 41 g/l Leucocytes for lyzosomal enzymes normal Urinary excretion of amino acids and acid mucopolysaccharides normal An electroencephalogram was taken on three different occasions at approximately 6-month intervals including tracings during photostimulation hyperventilation and slight dose Real sleep was not achieved despite adequate sedation All tracings were normal Ophthalmological examination normal Audiometry normal The vestibular function was not investigated Intelligence quotient 96 (Binet)

E Cardiac disease

because of multiple extrasystoles the patient was transferred to Medical Department B Rigshospitalet Copenhagen

Cardiovascular findings were unchanged Peripheral pulsation was normal Arterial blood pressure was 110/70 mmHg The chest roentgenogram showed a normal cardiac silhouette and pulmonary fields

The ECG was monitored continuously by telemetry during the whole of his 7 weeks stay in hospital Multiple extrasystoles probably of ventricular origin were present most of the time The percentage of extrasystoles varied from 0–84% usually being in the range of 30–40% A representative rhythm strip together with a 12 hour histogram is shown in Fig 4 Occasionally series of 3–5 extrasystoles were noted (Fig 5) Exercise was not consistently associated with



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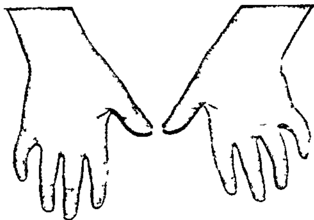


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Fig 3 Central defect of the hard palate surrounded by cicatricial changes after surgical intervention

pulmonary stenosis cardiac conduction disturbances of impulse formation and conduction are present (2) However the physical findings chest roentgenogram and electrocardiogram in our patient did not support the diagnosis of a valvular anomaly There were no lentigenes of the skin and the patient s hearing was normal

In neuromuscular diseases the myocardium is often affected and often disturbances of impulse formation and conduction are present (5) The normal size and shape of the heart in this patient together with an essentially normal ECG (*in the absence of extrasystoles*) and the absence of cardiac symptoms make the diagnosis of myocardiopathy unlikely

In certain muscular diseases as for instance the progressive muscular dystrophies the blood concentration of intracellular enzymes such as creatine phosphokinase aldolase lactic acid dehydrogenase and glutamate pyruvate transaminase are markedly elevated (10) In our patient the elevation of the serum level of creatine phosphokinase was only slight and intermittent and the levels of all the other enzymes mentioned were normal also during periods of muscular weakness The same apply to se potassium which together with the character of the episodes of muscular weakness speaks against a muscular disease associated with disturbances of potassium metabolism (*adynamia episodica hereditaria* familial periodic paralysis (1 2)) The urinary excretion of creatin and creatinine was normal Furthermore the electromyographic findings disclosed no abnormalities which also speaks against the possibility of myasthenia gravis which might have been suspected because of the intermittent character of the muscular weakness (6)

The possibility that the symptoms could be caused by an epileptic equivalent such as psychomotor epilepsy does not seem very likely The EEGs were all normal but unfortunately no curve was obtained during sleep However the symptoms were by no means characteristic of psychomotor seizures and the patient was fully conscious during the attacks

Concerning the other characteristics of our patient the reason for his dwarfism remains obscure Plasma levels of protein bound iodine and cortisol were normal as was urinary excretion of 17 ketosteroids and 17 ketogenic steroids Pituitary reserve of corticotropin was not decreased as judged by the metyraponetest Sella turcica was of natural shape and size and skeletal development was not retarded

The peculiar appearance of the patient s face has a certain resemblance to the syndrome of bird headed dwarfism (8) but other essential features of this syndrome are missing (especially mental retardation) Further this syndrome does not include any cardiac signs

The palatal defect the mandibular hypoplasia and the aplasia of some of the teeth were of no further help in the classification of the patient

In all the above mentioned syndromes a hereditary factor seems to play an important role The family history of our patient does not support this possibility The pregnancy was complicated by vaginal haemorrhage in the second month and the mother was treated with hormones This may in an unknown way have been of some importance for the pathogenesis of the developmental anomalies in our patient

It is tempting to suggest that the presence in a child of symptoms and signs involving a number of organs and tissues be due to a chromosomal anomaly Even if we have not been able to demonstrate one this remains a possible explanation of the findings on our case as current techniques only allow visualization of very gross abnormalities of chromosomal structure involving several thousand genes

SUMMARY

A description is given of an eight year old boy with extrasystoles, seizures of muscular weakness and multiple developmental anomalies (dwarfism scaphocephalic skull hypertelorism bilateral ptosis low set ears broad nose mandibular hypoplasia aplasia of a number of teeth defect of both the soft and os-

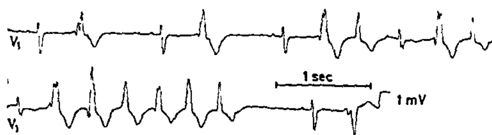


Fig 5 ECG sho
and s nal ectop

more extrasystoles than were present at rest A 12 lead ECG was essentially normal but sometimes a slight ST depression and inversion of T waves were present in leads V_1 through V_4 (Fig 6) On the assumption that his syncope and dizzy spell might have been caused by runs of extrasystoles a therapeutical trial was undertaken The following regimens tried in succession over a period of 7 weeks failed to reduce the number of extrasystoles practolol 225 mg/day propranolol 100 mg/day and procainamide 1 g/day N propyl ajmalinium bitartrate (Giulini) a new drug which is not yet commercially available in a dose of 20 mg/day did result in a reduction of the number of extrasystoles of about 75 % However because of lack of experience with this drug it was decided not to continue this therapy on a long term basis

He had no syncopes or dizzy spells during this admission He experienced four episodes of muscular weakness which were witnessed by the staff On one occasion he was not able to raise his arms on another while walking around in the ward he suddenly fell to the floor He was not able to rise without help He could walk but limped A considerable decrease of muscular strength in the right leg was clearly demonstrated when he tried to climb stairs He was fully conscious during these episodes

During such periods of muscular weakness which were not related to any changes in the ECG se LDH (including iso enzymes) se aldolase se creatinin phosphokinase and se potassium were normal The attacks would last for a few hours He had had these attacks since early childhood at intervals varying between 1–2 weeks to 1–2 months each episode lasting 1/2 hour to 2 days There was no constant relation to rest physical activity or meals To investigate the latter possibility further a glucose tolerance test including determinations of se potassium was performed and was found to be normal

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In Gorlin s Leopard Syndrome

Fig 6 12 lead ECG
Paper speed 50 mm

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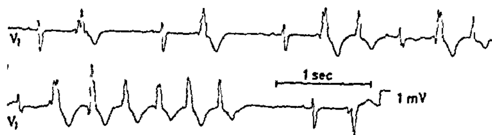


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The patient was discharged September 27th 1969 and has since then been seen in January and again in July 1970 in Paediatric Department G During this period the seizures of muscular weakness seem to have d creased His ECG was recorded by a portable taperecorder for 2 / hours in July 1970 On the average the number of extrasystoles was unchanged but for shorter periods of time more series of 2–6 extrasystoles were noted The child was still free of cardiac symptoms and had no syncop s Physical find ings were unchanged

DISCUSSION

The most outstanding features of the present case are extrasystoles, seizures of muscular weakness and multiple developmental anomalies in connection with a characteristic appearance These findings naturally lead one to consider the presence of a syndrome but we have not been able to find a similar com bination of symptoms in the literature

In a number of syndromes cardiac signs and symptoms are prominent (9) Holt Oram s syndrome is characterized by a combination of disturbances of cardiac rhythm atrial septal defect and skeletal defects of the upper extremities (5) In our patient there was nothing to suggest the presence of a septal defect and the roentgenogram disclosed no malformations of the skeleton

In Gorlin s Leopard Syndrome lentigo

Fig 6 12 lead ECG at rest Paper speed 50 mm/sec

pulmonary stenosis cardiac conduction disturbances of impulse formation and conduction are present (2) However the physical findings chest roentgenogram and electrocardiogram in our patient did not support the diagnosis of a valvular anomaly There were no lentigenes of the skin and the patient s hearing was normal

In neuromuscular diseases the myocardium is often affected and often disturbances of impulse formation and conduction are present (5) The normal size and shape of the heart in this patient together with an essentially normal ECG (in the absence of extrasystoles) and the absence of cardiac symptoms make the diagnosis of myocardiopathy unlikely

In certain muscular diseases as for instance the progressive muscular dystrophies the blood concentration of intracellular enzymes such as creatine phosphokinase aldolase lactic acid dehydrogenase and glutamate pyruvate transaminase are markedly elevated (10) In our patient the elevation of the serum level of creatine phosphokinase was only slight and intermittent and the levels of all the other enzymes mentioned were normal also during periods of muscular weakness The same apply to serum potassium which together with the character of the episodes of muscular weakness speaks against a muscular disease associated with disturbances of potassium metabolism (adynamia episodica hereditaria familial periodic paralysis (1 2)) The urinary excretion of creatin and creatinine was normal Furthermore the electromyographic findings disclosed no abnormalities which also speaks against the possibility of myasthenia gravis which might have been suspected because of the intermittent character of the muscular weakness (6)

The possibility that the symptoms could be caused by an epileptic equivalent such as psychomotor epilepsy does not seem very likely The EEGs were all normal but unfortunately no curve was obtained during sleep However the symptoms were by no means characteristic of psychomotor seizures and the patient was fully conscious during the attacks

Concerning the other characteristics of our patient the reason for his dwarfism remains obscure Plasma levels of protein bound iodine and cortisol were normal as was urinary excretion of 17 ketosteroids and 17 ketogenic steroids Pituitary reserve of corticotropin was not decreased as judged by the metyraponetest. Sella turcica was of natural shape and size and skeletal development was not retarded

The peculiar appearance of the patient s face has a certain resemblance to the syndrome of bird headed dwarfism (8) but other essential features of this syndrome are missing (especially mental retardation) Further this syndrome does not include any cardiac signs

The palatal defect the mandibular hypoplasia and the aplasia of some of the teeth were of no further help in the classification of the patient.

In all the above mentioned syndromes a hereditary factor seems to play an important role The family history of our patient does not support this possibility The pregnancy was complicated by vaginal haemorrhage in the second month and the mother was treated with hormones This may in an unknown way have been of some importance for the pathogenesis of the developmental anomalies in our patient

It is tempting to suggest that the presence in a child of symptoms and signs involving a number of organs and tissues be due to a chromosomal anomaly Even if we have not been able to demonstrate one this remains a possible explanation of the findings on our case as current techniques only allow visualization of very gross abnormalities of chromosomal structure involving several thousand genes

SUMMARY

A description is given of an eight year-old boy with extrasystoles seizures of muscular weakness and multiple developmental anomalies (dwarfism scaphocephalic skull hypertelorism bilateral ptosis low-set ears broad nose mandibular hypoplasia aplasia of a number of teeth defect of both the soft and os

seous palate, inward bending of the fifth fingers, single transverse palmar crease of both hands, and cryptorchidism) These findings suggest a specific syndrome, but no similar description was found in the literature The investigations disclosed no signs of either a chromosomal, a neuromuscular, or an endocrine disease

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CARBIMAZOLE TREATMENT IN EARLY PREGNANCY

Ultrastructural and Biochemical Observations on the Thyroid Glands of two Twin Fetuses

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Thyrostatic drugs are known to pass through th placenta in the human and in animals at least in late pregnancy (1–3 5 7 8) The effect of these drugs on the human fetus in the second trimester of pregnancy is little known although the onset of thyroid function occurs at the conceptual age of 10 to 11 weeks corresponding to a fetal crown–rump length (CR) of 60–65 mm (16 17) The treatment of choice in the pregnant women with thyrotoxicosis is therefore still a matter of debate (1–2 10 12 14) Accordingly it may be pertinent to present the findings in two twin fetuses on the rare occasion when a thyrotoxic woman on carbimazole was subjected to a therapeutic abortion in the 14–17th week of gestation

CASE REPORT

A 44 year-old married woman was subjected to a therapeutic abortion because of hyperthyroidism and weakness She became thyrotoxic at the age of 26 years and underwent then a subtotal thyroidectomy with clinical remission postoperatively She bore 4 normal children the last one born in 1961 In February 1969 she saw her gynecologist because of possible pregnancy her last menstrual bleeding occurring on November 10 1968 At the same time she complained of heart trouble The clinical examination disclo ed a blood pressure of 200/120 mm Hg and a cardiac arrhythmia The thyroid gland was enlarged. There was no exophthalmos but a starring expression She had fin er tremor The clinical diagnosis of thyrotoxi osis was verified by a PBI of 14 1 μg/100 ml (normal value during pregnancy 7–12 μg/100 ml) and a T resin uptake of 119% (normal range 80–120%) LATS was not detectable by the Mckenzie bio-assay An X ray of the chest was normal The ECG showed atrial fibrillation She was treated with carbimazole 15 mg three times daily for 14 days before the operation During the treatment the goitre decreased in size The PBI was 7 7 μg/100 ml and the T resin uptake was 99% at the time of the abdominal hysterotomy on March 24 1969 The twin fetuses of a CR of 120 and 105 mm and a weight of 105 and 81 g respectively showed no external abnormalities The thyroid glands were not conspicuously enlarged but at the excision the glands appeared more vascular and softer than those of other fetuses of the same size

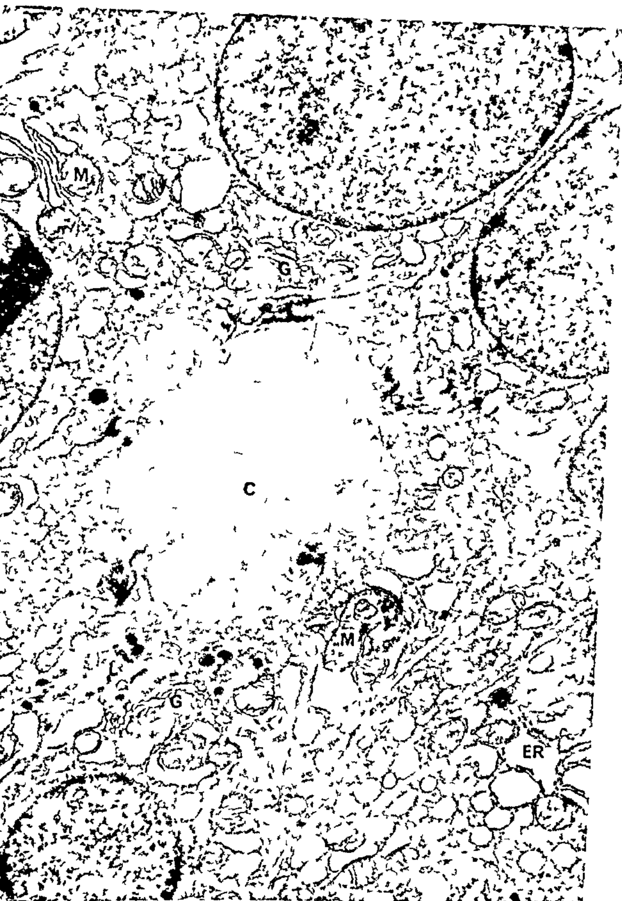
METHODS

The fetal thyroid glands were excised 1 hour after the operation One lobe from each gland was immediately immersed in a glutaraldehyde fixative and processed as described previously for light and electron microscopy (16) The second lobe was used for biochemical studies as described in detail in a previous paper (17) These lobes were cut in 4–5 pieces and incubated for 4 hours at 37 C in 2 ml of Eagle medium without leucine containing 100 μCi of H l leucine (spec act 15 9 Ci/mM) and 23 μCi of carrier free Na ^{125}I The tissues were weighed and the ^{125}I radioactivity was measured in the tissue and the medium The ratio of the ^{125}I radioactivity per mg tissue/^{125}I radioactivity per μl medium was calculated The soluble proteins were extracted with a phosphate buffer separated by means of linear sucrose density gradient centrifugation and analysed for ^{125}I and H radioactivities as described earlier (17)

RESULTS

Morphology

The description given below is not meant as a complete account of the morphology of the



/ Survey electron micrograph of a thyroid fol from the smaller twin fetus (CR 105 mm) The e lumen is filled with colloid (*C*) into which villi project from the apical cell surfaces The follicle cells have well developed rough surfaced endoplasmic reticulum (*ER*) and Golgi apparatus (*G*) Mitochondria (*M*) and lysosomes (*L*) are also seen × 8 000



Fig 2 Electron micrograph of the apical portions of two follicle cells (fetus CR 105 mm) Microvilli are seen projectin into the dense colloid (*C*) The cisternae of the rough surfaced endoplasmic reticulum (*ER*) contain a floccalent material In the cytoplasm a large number of small vesicles are seen as well as a few mitochondria (*M*) and lysosomes (*L*) 14 000

two fetal thyroids only those cell components that are of special significance in judging the effect of an enhanced TSH stimulation are dealt with The evaluation of the observations is based on the results obtained in a previous study on the thyroid of normal fetuses (16)

The light microscopic examination of the fetal thyroids showed that the epithelial cells were organized into groups separated by spaces containing connective tissue and capillaries Many of these groups appeared compact but in approximately 50% of the groups the cells were arranged concentrically around a lumen which was filled with a substance deeply stained by toluidine blue These microfollicles were considerably smaller than those of the adult human thyroid and had a proportionally smaller lumen

Electron microscopical survey pictures (Fig 1) revealed that the microfollicles were composed of cuboidal to columnar cells surrounding a lumen containing a homogeneous substance of high density The lumina were either clover leaf shaped or rounded The cell surfaces bounding the follicle lumina were provided with numerous long and slender microvilli (Fig. 2) In addition the apical region of some cells had rounded lumina which were bounded by a membrane projecting into the lumen in numerous microvilli and which contained a homogeneous dense substance Whether these intracellular lumina are true intracellular cavities or canaliculi extending from the follicle lumen has not been settled in the present study (For further discussion the reader is referred to Olin Ekholm & Almqvist 1970 (16)) No pseudopodia indicating phagocytosis of colloid were seen on the apical cell surfaces

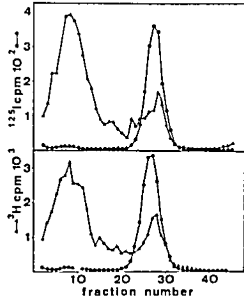


Fig 3 Sucrose density gradient centrifugation patterns of the soluble thyroid extracts from the twin fetuses after 4 hr *in vitro* labelling of the thyroid glands with $^{125}I^-$ and ^{3}H leucine *Top* fetus CR 105 mm and *bottom* fetus CR 120 mm The main radioiodine peaks represent thyroglobulin (17) Sedimentation is from left to right The gradient contains 5–20 % sucrose in 0 02 M sodium phosphate and 0 1 *M* KCl pH 7 4 Centrifugation for 220 min at 200 000 *g* Each fraction is plotted

The follicle cells (Fig 2) had a well developed rough surfaced endoplasmic reticulum the basal and middle cell regions were almost filled with large, rounded or elongated cisternae studded with ribosomes on their outer surfaces and containing a flocculent material of moderate density the apical cell zone contained numerous similar but smaller cisternae Free ribosomes, generally gathered in clusters were numerous The Golgi apparatus was well developed and was characterized by a large number of small vesicles Similar smooth surfaced vesicles with a content of low to moderate density were also found in considerable numbers in the apical cell zone The same zone contained many round or oval, very dense bodies interpreted as lysosomed Very rarely structures were observed in the apical and middle cell zones that could be interpreted as colloid droplets they were rounded, membrane-bounded bodies, generally somewhat larger than the biggest lysosomes, and containing a homogeneous substance of high to moderate density

Biochemistry

The weight of one thyroid lobe in the twin fetuses was 28 5 mg and 25 2 mg respectively (normal range for total thyroid weight 20–70 mg at CR 120 mm (16, 18)) When the lobes were incubated for 2 hours in the presence of $^{125}I^-$ and ^{3}H-leucine they accumulated $^{125}I^-$ from the medium The ^{125}I tissue over medium ratio was 19 1 and 24 4 respectively 88–92% of the ^{125}I radioactivity (total $3\,5\text{–}5\,0 \times 10^6$ cpm) could be extracted After extensive dialysis 2 7–7 4% of the ^{125}I radioactivity was left in the dialysis bag and considered to be proteinbound

Separation of the soluble proteins by ultracentrifugation on sucrose density gradients resulted in a radioactivity peak for both ^{3}H and ^{125}I in the thyroglobulin region ^{3}H labeling was also found in slow sedimenting proteins (Fig 3)

DISCUSSION

There are only two previous reports on the effects of thyrostatic agents on the preterm human fetal thyroid gland Davis & Forbes (4) reported histological signs of hyperactivity in the thyroid gland of a 24 week old fetus of a thyrotoxic woman treated with propylthiouracil Freiesleben & Kjerulf Jensen (7) mentioned an increased thyroid weight in a fetus of a crown–heel length of 23 cm fetal age 19 weeks whose mother was treated with propyl thiouracil for thyrotoxicosis The present case report of two twin fetuses of a CR of 105 and 120 mm fetal age 14–17 weeks is the first presentation of ultrastructural and biochemical studies in human fetuses subjected to thyrostatic drugs given to the mother In contrast to the earlier reports the present investigation showed a normal thyroid weight with respect to fetal size and the light and electron microscopic morphology of the thyroid was normal as compared with our observations on normal

fetuses of corresponding age (16) Thus there were no morphological features indicating a pathologically augmented TSH-stimulation Pseudopodia on the apical cell surface and numerous colloid droplets indicative of an increased hormone release were not observed dilatation of the cisternae of the endoplasmic reticulum and hyperplasia of the Golgi apparatus alterations which are considered to reflect an increased protein synthesis (11 18) could not be established The biochemical *in vitro* studies showed a considerable incorporation of ¹²⁵I into thyroglobulin indicating that the maternal treatment with carbimazole did not cause prolonged inhibition of the organification of iodine in the fetal thyroid gland

The normal weight, morphology and incorporative capacity of iodine could possibly be due to the relatively short duration of the carbimazole treatment of the mother However this interpretation does not seem very likely since the treatment was efficient as documented by the reduction of her PBI from 14 1 to 7 7 μg/100 ml during the 14 day period There are two other more plausible explanations of the lack of effect of the carbimazole treatment in our study One concerns the efficiency of the fetal pituitary thyroid feed back system and the other the permeability of the placenta for thyrostatic drugs at this stage of fetal development

In a previous paper we showed that the TSH content of the human fetal pituitary starts to increase at a CR of 60 mm (15) Greenberg et al (9) found that the TSH and free thyroxine concentrations in the fetal plasma are rising from approximately the same stage of development These findings demonstrate that TSH is secreted and indicate that the thyroid is sensitive to TSH On the other hand they do not answer the question whether the fetal pituitary at this stage of development is sensitive to changes in the plasma level of free thyroxine Indeed the data of Fisher et al (6) suggest that the fetal thyroid pituitary feed back system is not functioning until the 18th to 22nd week of gestation Thus the lack of effect of carbimazole in our fetuses in the 14–17th week of gestation may be explained by an immature feed back system

It is well established both in animals and in man that thyrostatic drugs pass through the placenta at term (1–3 5 7 8 13) but nothing is known about this passage in the second trimester As we know from a previous study (15) that propylthiouracil inhibits iodine organification in the human fetal thyroid even from a CR of 65 mm it cannot be excluded that the absence of visible carbimazole effects in the present study at least partly is due to a restricted placental permeability for this drug

Our findings of normal structure and normal capacity of iodine incorporation *in vitro* in the fetal thyroid in the 14–17th week of gestation after a short treatment of the mother with high doses of carbimazole do not contravene the suggestion of Herbst & Selenkow (12) that thyrotoxicosis during pregnancy can be treated with small doses of thyrostatic agents However the maternal fetal and thyroid pituitary relationships at different stages of development are far from unravelled Further experimental and clinical studies are necessary before the optimal treatment of thyrotoxicosis during pregnancy should be formulated

SUMMARY

This paper presents ultrastructural and biochemical studies on the thyroid glands from two twin fetuses of a CR of 105 and 120 mm whose thyrotoxic mother was treated for 14 days with carbimazole The light and electron microscopic appearance of the fetal thyroids corresponded to that normally seen at this stage of development No morphological signs of excessive TSH stimulation were detected The fetal thyroid glands incorporated radioiodine and labelled leucine into thyroglobulin *in vitro* These normal findings during thyrostatic treatment of the mother are discussed and related to the efficiency of the fetal pituitary thyroid feed back system and to the passage of thyrostatic agents through the placenta

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SHORT TERM DIFFERENCES OF INFANT DEVELOPMENT IN NURSERY HOMES AND IN PRIVATE FAMILIES

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Medical practice has changed in two major respects as a result of the focus on maternal deprivation The dramatic research results from several countries published by R A Spitz (11 12) in the forties and the extensive overview provided by John Bowlby (1) for the World Health Organization contributed to a sharp decline in the willingness to separate mother and child during the first years of life Secondly they sparked efforts towards creating more personalized stimulating and emotionally warm wards for those children who were being deprived of their parents care

The key term maternal deprivation has remained controversial however

In the psychologically unhealthy institutions studied by Spitz, Dennis and Narajan and others all of the important sensory modalities were understimulated It may be appropriate therefore to refer to the disturbances found among institutionalized children (at least in those cases where separation from the mother *occurred* before the age of 6 months) as perceptual deprivation including tactile and kinesthetic rather than using the too-broad and yet too-specific term maternal deprivation (3)

In his first research report concerning this problem Spitz stated

Two factors, both already stressed by Durfee and Wolf are made responsible by most of the authors for the psychological injury suffered by these *children* First lack of stimulation The worst offenders were the best equipped and most hygienic institutions which succeeded in sterilizing the surroundings of the child from germs but which at the same time sterilized the child s psyche Even the most destitute of homes offers more mental stimulation than the usual hospital ward.

Second the presence or absence of the child s mother Stimulation by the mother will always be more intense than even that of the best trained nursery personnel Those institutions in which the mothers were present had better results than those where only trained child nurses were employed The presence of the mothers could compensate for numerous other shortcomings (11)

While Spitz concluded that the second factor was the most consequential no other research reports have been found by this author that provide solid empirical proof for that conclusion Its widespread acceptance is based on theoretic considerations as much as on empirical information The psychoanalytic thesis that perception is a function of libidinal cathexis certainly necessitated assigning more weight to maternal than to perceptual deprivation during the first year of life (5)

The nature of this conflict is such that experimental efforts to provide conclusive empirical evidence would be ethically unacceptable Answers to secondary questions raised by the central conflict can be found however The present investigation is an attempt to measure short term developmental effects of deprivation of *continuous* and *emotionally intense* parental care An experimental group of 22 institutionalized infants was given the Hetzer Buhler baby tests at 6 and 8 months of age and compared to a control group of infants in private families Attempt was made to control for quantity and in some respects for quality of parental care Such measurement was possible in Oslo Norway where a number of small

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were placed in nursery homes because their mothers were unwed had to work and could not manage to take care of their child Four other babies were like wise born to unwed mothers but in these cases the public Child Welfare Agency had decided the mother unfit to be responsible for her child (These were children b c e and m) Reasons for placement of the remaining eight babies could not be obtained from official records This implies the reason could not be the same as for the four previously mentioned and communication from nurses involved in their care suggested these babies were placed by unwed mothers who materially were unable to take care of them

The second sample was composed of babies living with their own mothers These subjects were selected at a public Well Baby Clinic in Oslo Only two of these mothers were employed and both had adequate baby sitting facilities

The same four criteria were followed in the selection of both samples health age sex and social class background In both groups the first 22 children to satisfy the criteria were selected for testing The health criterion involved that the baby should not have suffered any prolonged or serious disease or have been hospitalized for periods exceeding one week total Neither should he appear retarded in any way or suffer impairment of any sense organ Child h in the nursery sample was a borderline case in this respect He was hard to communicate with at six months and it was suspected that his hearing might be impaired. Medical examinations were negative however and by 8 months he was alert and outgoing

The babies were all to be 6 months at the time of the first testing Computation of average age for family children gave $\bar{x}=6\ 00$ with $s=7$ and for nursery children $\bar{x}=6\ 02$ with $s=12$ At second testing the ages were $\bar{x}=7\ 28$ with $s=8$ for family children and $\bar{x}=8\ 01$ with $s=12$ for nursery children The sex distribution was 10 girls to 12 boys in the private families and 11 to 11 in the nursery homes

It was impossible to obtain a measure for social class background which would include all the babies in the nursery homes The official records were entirely lacking in information of occupational or educational status and the investigator had to rely on information given by nurses and administrators who had contact at some point with the mothers of these babies The estimates were consistently given as predominantly working class some lower middle class According to this standard the children from private families were selected 8 from social stratum V 11 from stratum IV and 3 from stratum III Assignment to social stratum was done on the basis of fathers occupation in correspondence with the five point scale developed by Svalastoga in his major work on social stratification in Scandinavia (13)

The reality of the assignment to social class was illustrated by the variety of life situations incidentally noted within the sample child F was living with her unwed mother in grandparents apartment L and ved with their teen age mothers mostly in grandparents apartment with father largely absent and O lived with her mother and her mothers daughter of an earlier marriage the husband being recently evicted by court order for alcoholism

REARING PRACTICES

Quantitative aspects Most of the mothers in the private homes had more than one child and none had outside help in the house The ratio of nurses to children in the nurseries was better than 1 to 3 and the amount of work other than caring for the children considerably less than in a home Each nurse had a couple of children as hers so that each child normally was handled by a small number of nurses and not rotated among the whole staff It is therefore not necessarily true that an average child would spend more time in direct contact with his parents than a child in a nursery home would spend with two particular nurses

With respect to specifics of daily routine the nurseries and private families compared as follows for children 7 months of age Number of hours awake per day varied from 6 to 12 in private homes with 8 and 9 hours being most common The nurseries were more uniform with most children awake approximately 8 hours daily All children were fed four times a day except three of the family children who had only three meals Each meal was estimated to take 10 to 15 min by the mothers this was also the approximate time for a meal in the nurseries Again the variation was somewhat greater in the families one mother estimated 5 to 30 min and one 30 to 60 min No meal in the nurseries was observed to last longer than 20 min

Meal times were said to be regulated to some extent by the children themselves in all families except one Probably the extent to which this happened was smaller in the nurseries The meals there occupied a full hour and within that time period there was no set order A child showing clear signs of hunger would be fed first.

Diapers were changed four times daily in all families except four who had five changes and two who had six Diapers were changed in connection with every meal in the nurseries making four changes the basic norm Occasionally a child would be changed if he showed discomfort between meals or he would be changed if he was so wet as to be unpleasant to handle

Ten of the mothers said they usually picked their baby up if he was crying. Four of these added if he is not crying to be picked up" Four mothers gave a clear no to the question two said, after a while and one said only if in pain" Fear of spoiling the baby was given as reason for other than affirmative answer The same attitude was expressed by several nurses The situation was different in the nurseries however because of their more clearly defined wake and sleeping hours The investigator often heard babies cry during sleeping hours In about 50% of these instances a nurse

nursery homes have been changing their organization, staffing, and child caring procedures through the fifties and sixties towards a closer approximation of family type care in a varied and stimulating environment They do, however, maintain the following characteristic which makes them inadequate with hespect to continuous parental care Those close relationships that are established are regularly broken because virtually all the nurses are doing their internships and never stay longer than one year in one institution

It was decided to test the infants at 6 and 8 months of age so as to obtain a measure of development during that period Texts in child psychology (8) generally list 6 months as the age when an infant normally starts differentiating between individuals John Bowlby (2) states that infants who lose their mothers after this point fret, while those who lose them earlier do not Therefore, the 2 months after 6 months of age were expected to be particularly sensitive to discontinuous and less emotionally intense parental care

METHOD

The private families were visited twice The first visit lasted for little more than one half hour and was occupied with testing the 6 months old infant The second visit lasted close to one hour and included an informal interview with the mother Some notes were taken during the talk and the following questions were always included although in varying order and with follow up questions to clarify specific points

How many times a day is your baby fed?
How long does the feeding take?
How are the feeding times determined—does the child influence them?
How often (day average) do you change his diapers?
Is the baby picked up when he is crying?
How often do you pick him up for other reasons?
How long is he awake each day?
Where does he spend the time he is awake?
Are there people in the same room with him? Who?
How is he placed when awake?
Is he able to see what is happening in the room?
How many and what toys does he have?

The nursery homes provided opportunity for direct observation of childcaring practices Prior to testing the investigator spent hours most days of the week for 2 months in nursery home I Ostensibly he was practicing and learning how to use his tests This provided the advantage normally associated with participant observation of becoming an accepted part of the milieu and being able to observe its normal functioning Notes were taken during this time immediately after leaving the nursery home

Particular interest focused on the character of the relationships between nurse and child in feeding and other situations involving handling Easily objectifiable observations such as number of feedings were checked with the nurses for accuracy

The basis for description of the other nursery homes was not equally thorough Nurseries III and IV were visited ten times each and found virtually identical to nursery I with respect to the characteristics described in this report Nurseries II and V were visited only three times each While this was insufficient for making any positive statement as to identity of procedure no marked difference in character was noted by the investigator Nursery II differed in being slightly disorganized due to shift of chief administrator at the time of the investigation

The children were distributed as follows Nursery I a b c d e f g h i j k l Nursery II m n Nursery III o p q Nursery IV r s t u and Nursery V v Only three subjects were therefore placed in insufficiently characterized environments The great similarity in character of the nursery homes was further collaborated by the circumstance that they all were responsible to the same government agency for the care of the children The administrators and pediatricians at the different homes maintained a high level of professional communication

The infants were given the Hetzer Buhler developmental tests (7) at ages 6 and 8 months Although these tests are not standardized for Scandinavian countries they are the ones most widely used and they have been shown to differentiate well between children from different environments (9 10)

The procedure of testing and the computation of results followed closely that outlined in the English edition of 1935 with three minor exceptions Item 2 series VI item 7 series VI and item 5 series IX were omitted because of technical difficulties In tabulation of the results these three items were counted as passed if one or more other items in the same series was passed

For the family children the tests were administered in the home at an hour when the infant was awake and active The nursery children were given the tests in or around their beds at wake hours A happy and interested atmosphere was secured for every test period

SUBJECTS

Two samples each including 22 infants were selected for the investigation One group came from five different nursery homes in the Oslo area Average age upon placement was 3 weeks and all subjects had been in the same nursery home from the time they were first placed there Ten of these babies

were placed in nursery homes because their mothers were unwed had to work and could not manage to take care of their child Four other babies were like wise born to unwed mothers but in these cases the public Child Welfare Agency had decided the mother unfit to be responsible for her child (These were children b c e and m) Reasons for placement of the remainin eight babies could not be obtained from official records. This implies the reason could not be the same as for the four previously mentioned and communication from nurses involved in their care suggested these babies wer placed by unwed mothers who materially were unable to take care of them

The second sample was composed of babies living with their own mothers These subjects were selected at a public Well Baby Clinic in Oslo Only two of these mothers were employed and both had adequate baby-sitting facilities

The same four criteria were followed in the selec tion of both samples health age sex and social class background In both groups the first 22 children to satisfy the criteria were selected for testing The health criterion involved that the baby should not have suffered any prolonged or serious disease or have been hospitalized for periods exceeding one week total Neither should he appear retarded in any way or suffer impairment of any sense organ Child h in the nursery sample was a borderline case in this respect He was hard to communicate with at six months and it was suspected that his hearing might be impaired. Medical examinations were nega tive however and by 8 months he was alert and outgoing

The babies were all to be 6 months at the time of the first testing Computation of average age for family children gave $\bar{x}=6.00$ with $s=7$ and for nursery children $\bar{x}=6.02$ with $s=12$ At second test ing the ages were $\bar{x}=7.28$ with $s=8$ for family chil dren and $\bar{x}=8.01$ with $s=12$ for nursery children The sex distribution was 10 girls to 12 boys in the private families and 11 to 11 in the nursery homes

It was impossible to obtain a measure for social class background which would include all the babies in the nursery homes The official records were en tirely lacking in information of occupational or educa tional status and the investigator had to rely on information given by nurses and administrators who had contact at some point with the mothers of these babies The estimates were consistently given as pre dominantly working class some lower middle class According to this standard the children from private families were selected 8 from social stratum V 11 from stratum IV and 3 from stratum III Assignment to social stratum was done on the basis of father's occupation in correspondence with the five point scale developed by Svalastoga in his major work on social stratification in Scandinavia (13)

The reality of the assignment to social class was illustrated by the variety of life situations incidentally noted within the sample child F was living with her unwed mother in grandparents apartment L and M both lived with their teen age mothers mostly in grandparents apartment with father largely absent and O lived with her mother and her mother s daughter of an earlier marriage the husband being recently evicted by court order for alcoholism

REARING PRACTICES

Quantitative aspects Most of the mothers in the private homes had more than one child and none had outside help in the house The ratio of nurses to children in the nurseries was better than 1 to 3 and the amount of work other than caring for the chil dren considerably less than in a home Each nurse had a couple of children as hers so that each child normally was handled by a small number of nurses and not rotated among the whole staff It is ther fore not necessarily true that an average child would spend more time in direct contact with his parents than a child in a nursery home would spend with two particular nurses

With respect to specifics of daily routine the nurseries and private families compared as follows for children 7 months of age Number of hours awake per day varied from 6 to 12 in private homes with 8 and 9 hours being most common The nur series were more uniform with most children awake approximately 8 hours daily All children were fed four times a day except three of the family children who had only three meals Each meal was estimated to take 10 to 15 min by the mothers this was also the approximate time for a meal in the nurs ries Again the variation was somewhat greater in the families one mother estimated 5 to 30 min and one 30 to 60 min No meal in the nurseries was ob served to last longer than 20 min

Meal times were said to be regulated to some extent by the children themselves in all families except one Probably the extent to which this happened was smaller in the nurseries The meals there occupied a full hour and within that time period there was no set order A child showing clear signs of hunger would be fed first

Diapers were changed four times daily in all familes except four who had five changes and two who had six Diapers were changed in connection with every meal in the nurseries making four changes the basic norm Occasionally a child would be changed if he showed discomfort between meals or he would be changed if he was so wet as to be unpleasant to handle

Ten of the mothers said they usually picked their baby up if he was crying. Four of these added if he is not crying to be picked up" Four mothers gave a clear no to the question two said after a while and one said only if in pain" Fear of spoiling" the baby was given as reason for other than affirmative answer The same attitude was ex pressed by several nurses The situation was diff rent in the nurseries however because of their more clearly defined wake and sleeping hours The in vestigator often heard babies cry during sleeping hours In about 50% of these instances a nurse

Table 1 *Developmental Quotients*

	Family children				Nursery children		
	DQ at 6 months	DQ at 8 months	Change in DQ		DQ at 6 months	DQ at 8 months	Change in DQ
A	112	116	4	a	112	113	1
B	136	143	7	b	125	120	− 5
C	127	119	− 8	c	123	123	0
D	130	130	0	d	122	118	− 4
E	132	142	10	e	115	107	− 8
F	132	149	17	f	141	129	−12
G	121	131	10	g	111	107	− 4
H	130	125	− 5	h	117	129	12
I	124	126	2	i	130	117	−13
J	108	122	14	j	138	112	−26
K	121	123	2	k	115	100	−15
L	135	147	12	l	121	114	− 7
M	138	139	1	m	120	108	−12
N	125	124	− 1	n	122	122	0
O	138	134	− 4	o	133	144	11
P	124	136	12	p	118	120	2
Q	127	117	−10	q	136	145	9
R	129	124	− 5	r	115	104	−11
S	127	127	0	s	122	124	2
T	117	124	7	t	121	117	− 4
U	121	133	12	u	149	145	− 4
V	114	120	6	v	126	110	−16
	2 768	2 851	83		2 732	2 628	−104
$\bar{x}$ =	125 8	129 6	3 8	$\bar{x}$	124 2	119 5	− 4 7
=	8 2	9 8	7 5	s =	9 9	12 7	9 3

All calculations from these values done with 42 degrees of freedom

would come in and lift the baby up change him or put him in a reclining infant seat for a while This however was true only for babies 5 months or older *The younger infants were normally left* to cry by themselves

During wake hours all children in both samples had one or more attentive adults in the same room with them Normally the babies were on the floor sometimes in a chair or an infant seat placed in their bed Six family children were reported to spend much time in a baby carriage A similarly small number of the nursery children would spend greater portions of their wake hours reclined in beds or carriages Except in these instances all babies were able to look around at what was happening in the room

During their seventh month the nursery children would spend one or two hours daily moving around each other on a rug No such experience was available to the family children All children had a number of adequate toys to manipulate

Qualitative aspects The following observations were done with age group 6 to 8 months No systematic observation was done with younger children The ratio of nurses to babies was smaller for the younger ones and the care they received was therefore probably less personal

With no exceptions the nursing staffs expressed in their work a great fondness for the infants Very few instances of impersonal or mechanical treatment were observed The nurses were most often *talking* to the infants when diapers were changed and the feeding situation involved good contact with the child regulating rate of intake and in many instances following the spoon or cup with his hands When babies were carried around or held on the lap the situation was normally characterized by delight in simple communication such as giving and taking toys exchanging smiles and babytalk

These observations could not be made comparatively There were however two important differences between relationships of mother and child and those of nurse and child The first concerns the intensity of love feelings on the part of the mother figure When nurses finished their employment after one year they expressed sadness to leave their children but their sadness was of course in no way comparable to that of a mother taking final leave of her child

The children who lost their nurses were observed to be less active and communicative and to eat less Often they were visibly unhappy for weeks This constituted the second main difference between living in a nursery and in a home loss and replacement of

the main parental figure was a regular fact of life in the nurseries

RESULTS

The measured developmental quotients are shown in Table 1 At 6 months a two-tailed t test gave probability greater than 0 5 that the two groups belonged to the same population with respect to over all level of development At 8 months of age the family children scored very much higher than the nursery children and the two-tailed t test gave a probability of less than 0 01 that the two groups still could be seen as belonging to the same statistical population When a two-tailed t test was applied to the change in development the probability was shown to be smaller than 0 002 that the two groups belonged to the same population

The Hetzer Buhler tests measure development in six different areas and this allows one to construct developmental profiles to show relative development of different abilities Fig 1 shows average developmental profiles for the two groups at ages 6 and 8 months The family children are shown to be approximately 2 weeks ahead in manipulation at 6 months of age the other areas tested being equally well developed in the two groups By 8 months of age the family children are almost 5 weeks ahead in manipulation 4 weeks advanced in mental production and 2 weeks in bodily control social reactions and learning

Some investigators have suggested that retardation in development of institutionalized infants may be due solely to lack of opportunity for specific physical exercises (4) If such was the case one would expect to be able to locate the relatively bad test results of the nursery children to a set of test items—mainly in the area of bodily control—with which they performed very badly In the present investigation there is some tendency to clustering of bad results in the area of manipulation but none in bodily control When the individual test items were compared one by one the better results of the family children

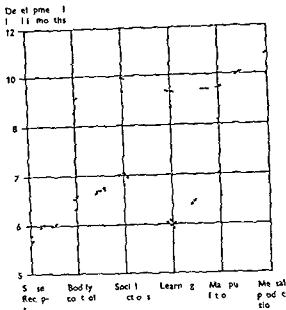


Fig 1 Average developmental profiles at 6 and 8 months --- family children nursery children

at age 8 months were spread rather evenly over most items

DISCUSSION

The sex distribution was slightly uneven in the two groups The family children had one boy more and one girl less than the nursery children Average change in DQ among the family children was +21 for boys and +62 for girls In the group of nursery children the average change was −57 for boys and −47 for girls Since the girls had a more favorable development in both groups the uneven sex distribution should have diminished rather than increased the difference in development between the two samples

The information obtained about parental background does not suggest as likely any genetic advantage on the part of the nursery sample The remarkably similar scores of the two groups at age 6 months therefore suggest that they may have developed at similar rates up to that point

Explanation of the difference in development

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situation in the nurseries as compared to the private families The emotional bonds between infant and parental figure appeared less intense and there was a lack of continuity of the relationship

At 6 months the probability was greater than 0 5 that the two groups belonged to the same population The nursery group showed a considerable relative retardation in the 2 months thereafter It is suggested that the 6 first months of an infant s stay in a nursery be sufficient for arrangement of adoption or sufficient improvement in parent(s) life situation for them to take care of the child

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can hardly be based on an assumption of a generally accelerated development—genetically based—of the family children and a genetically based decelerated development of the nursery children Since much work has shown such acceleration and deceleration to be expressed considerably earlier than 6 months (6), one should also have to assume a *considerably* higher—genetically or prenatally based—starting position for the nursery children This is highly unlikely on the basis of the data presented

It is noteworthy that institutions which consciously attempt to maintain a personalized and warm atmosphere may succeed equally well as a family in bringing children up to 6 months of age with respect to the many aspects of development measured by the Hetzer-Buhler scales This differs from earlier authoritative reports on early effects of institutionalization (6) Gesell & Amatruda reported diminished interest and reactivity and reduced integration of total behavior ' before 12 weeks of age Before 16 weeks they listed beginning of retardation evidenced by disparity between exploitation in supine and in sitting positions This should all show up in a Hetzer Buhler test which is very similar to the Gesell test for ages under one year

It is equally noteworthy, however, that this general warmth of the environment was not able to maintain an equally favourable development past six months of age The average developmental quotient was actually lower at 8 months than it was at 6 for the nurseries while a marked improvement had taken place in the families

Aside from the virtual absence of observed differences in amount and variation of tactile kinesthetic, and visual experiences, two specific aspects of the results strongly suggest that the significantly more favorable *development of* the family children was due to characteristics of the relationships between infants and parental figure rather than to *understimulation* of sensory modalities When test results were broken down item by item it turned out that the retardation of the nursery children was spread evenly over all items and all developmental categories Sensory deprivation in particular might have been expected to show up in the form of inadequate performance on a smaller number of item tests Secondly, Table 1 shows that the variability of scores increased *dramatically (75 %) for the nursery children and* much less (27 %) for family children over the two month interval when s is used as a measure Such dramatic increase in variability could be a result of the discontinuous and less emotionally intense parental care which affected the nursery children at different times and to various degree Possible sensory poverty of the environment should influence all nursery children more uniformly

Two policy recommendations follow from these findings Nursery homes should terminate their care for infants by the time they reach 6 months of age Adoption should either have been arranged by that time or the child s parent(s) should be aided financially and with housing and day care sufficiently to enable them to care for their own child Secondly nurses and trainees should be assigned babies in such a way as to assure each infant of a continuous relationship to his caretaker(s) while he is *placed in the institution*

SUMMARY

The investigation was done to determine the degree to which infant homes designed to be modern and humanized are successful in rearing infants when compared to private families Twenty two nursery children and twenty two family children were given the Hetzer Buhler developmental tests at ages 6 and 8 months The two samples were controlled for health, age sex and social background

Characterization of the social environment was made on the basis of participant observation home visits and informal interviews A remarkable similarity was found in the living environment for all the babies There were only two marked differences between the life

situation in the nurseries as compared to the private families The emotional bonds between infant and parental figure appeared less intense and there was a lack of continuity of the relationship

At 6 months the probability was greater than 0 5 that the two groups belonged to the same population The nursery group showed a considerable relative retardation in the 2 months thereafter It is suggested that the 6 first months of an infant s stay in a nursery be sufficient for arrangement of adoption or sufficient improvement in parent(s) life situation for them to take care of the child

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STUDIES ON THYROID PROTEINS IN CHILDHOOD GOITRE

P OLIN

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Congenital goitre may be caused by a block in the synthesis of thyroid hormones, e g in the step of iodide concentration by the thyroid gland, the step of oxidation of iodide and subsequent organification of the ion, or at the level of formation of iodothyronines from iodotyrosines These postulated defects in the synthesis of thyroid hormones have been deduced from clinical data and indirect biochemical evidence (18) Direct measurements of the concentrations of the enzymes which are thought to participate in these reactions have very rarely been performed Alternative explanations for the pathogenesis of the congenital goitres have been sought in the synthesis of the iodinated proteins mainly thyroglobulin in the thyroid During recent years there have been several reports regarding the presence of abnormal proteins such as thyralbumin and iodinated particle bound proteins in congenital goitres from sheep (4), cattle (16) and from some patients with congenital goitres (2, 6 8)

Biopsy studies performed at an early age before the appearance of nodules in the gland may give a more reliable picture of the pattern of iodoproteins in the goitre than the analyses on nodular goitres which hitherto have been presented This preliminary report deals with studies on thyroid proteins in two children with a possible defect in the organification of iodine and a child with goitre associated with iodine deficiency

CASE REPORTS

Case 1

This patient was an 18 day old boy with a symmetrical diffuse enlargement of the thyroid gland His mother and maternal grandmother had goitres Deafness was not present in the family The parents were unrelated and the boy was the first child of a 36 year-old woman who underwent surgery for myoma of the uterus and appendectomy in the third month of gestation She was treated with allyl stranol and hydroxyprogesterone for imminent abortion in the fourth month of gestation The child was born at Molndal Hospital after 38 weeks of gestation Birth weight was 3 700 g length 52 cm At birth an enlarged thyroid gland was noted and the child was transferred to the pediatric ward The boy was jittery during the first days of life Laboratory data including lumbar puncture calcium and magnesium in serum and repeated blood glucose measurements were essentially normal The neurological symptoms subsided in a few days when the child was on iv nutrition and oral calcium substitution The skeletal maturation was slightly retarded the caudal ossification centre of the femur measured 2 mm as compared with the normal full term value of 5 mm

The clinical studies of the thyroid function are presented in Table 1 Biopsy for the biochemical analyses was performed on day 18 Treatment with thyroxine was then started The gland diminished in size and the child s growth and development have been normal during the one year follow up

Case 2

A 4 year old girl had a goitre with hypothyroidism which was diagnosed at the age of 16 months Heredity for thyroid disorders was present The maternal grandfather s mother had a thyrotoxicosis and the maternal grandmother had a goitre since age 12 years On the maternal side there were also diabetes mellitus and allergic disorders The paternal grandmother had a juvenile goitre The grandmother s brother also had a goitre of unknown etiology No overt hypothyroidism

Table 1 *Clinical studies of thyroid function*

	Case 1	Case 2	Case 3		Normal range
Sex	male	female	female		
Age at biopsy	18 days	4 years	9 years		
Height cm		101 6	150 3		
Weight kg		15 1	42 6		
Thyroid function tests					
PBI µg/100 ml	3 7[a]	3 1[b]	6 5		4–8
TSH µU ml by radioimmunoassay	98→3 6[c]		290		<40
Radioiodine uptake % isotope and time also stated	35 [1] I 74 min	+[b d]	45 [131]I 4 h 50 [1 2]I 0 min		20–50
Perchlorate discharge % of thyroidal [13]I	30	+[b d]	0		<20
Urinary iodine µg/24 h			26		100–200
Thyroid antibodies					
Thyroglobulin tanned red cell agglutination		Negative[b]	1/1 600	1/3 200	<1/100[f]
Thyroid cytoplasm immunofluorescence		Negative[b]	1/25		<1/20
Complement fixation			Negative		Negative
Cytological examination	Signs of TSH stimulation no signs of thyroiditis		No signs of thyroiditis		
Pathological examination		Colloid goitre			
Audiometric test	Normal	Normal	Normal		

[a] On day one of life normal range 8–12 µg/100 ml at 24 h The mother s PBI was 8 3 µg/100 ml
[b] At the age of 16 months before thyroxine therapy
[c] After 3 weeks on thyroxine 50 µg/day
[d] Semiquantitative data utilizing [1] I
[e] At 15 months of age before treatment At the time of biochemical study the biopsy showed histological signs of an inactive gland with flattened epithelial cells and follicles of varying size (Dr B Robertsson)
[f] Normal value for adu ts

was known in any of the families and there was no deafness nor any consanguinity

The mother was treated with hydrochlorthiazide during the pregnancy because of edema After 36 weeks twins were born in a normal delivery The patient s birth weight was 2 480 g length was 49.5 cm The dizygotic twin (as judged by different blood groups) weighed 2 340 g length 47 cm When the patient was 2 months of age a swelling of her neck was observed by the mother The motor development of the patient was somewhat slower than that of her twin sister She did not walk at the age of 16 months

At the age of 15 months the child was admitted to the Department of Pediatric Surgery Kronprinsessan Lovisa s Barnsjukhus Stockholm because of a suspected thyroid tumour Surgical biopsy showed colloid rich thyroid tissue The child was transferred to the pediatric ward for further investigations The physical examination showed a girl of normal stature at the 50th percentile The skin was pale but the child was not distinctly hypothyroid The thyroid gland was prominent mainly the right lobe where the surgical biopsy had been performed The surface was smooth and the gland was considered to be diffusely enlarged She had 7 deciduous teeth (normal value at 16 months 10±6 $\bar{x}$±2 SD) The thyroid function te ts are given in Table 1 The biochemical techniques used in this paper were not available and the child was started on thyroid hormone During the first 6 months she received only 20 to 40 mg daily of desiccated thyroid In this period she did not increase in length She was then put on thyroxine 0 1 mg daily with rapid improvement The size of her thyroid gland decreased and she started to grow She started to walk at the age of 23 months Her psychomotor development was in the low normal region at age 3 5 years Her audiogram was normal The thyroid gland was still palpable at 4 years in spite of an adequate substitution therapy as judged by normal growth rate normal levels of protein bound iodine (PBI) and thyroid stimulating hormone (TSH) Percutaneous thyroid biopsy under general anaesthesia was performed for biochemical and histological analyses during thyroxine treatment before and after 6 days of treatment with TSH

Case 3

A 9 year old girl was admitted after having had a goitre for 1 month Her maternal grandmother had an adenomatous goitre A paternal female relative had an enlarged thyroid gland There was no known deafness nor any consanguinity in the family

The patient was the first of two children Her birth weight was 5 0 kg Pregnancy and the neonatal period were normal The past history was uneventful The psychomotor development was normal She was

Table 2 *Distribution of ^{125}I after in vitro incubation for 24 h of the thyroid biopsy specimens in the 3 patients and in human fetal thyroid glands incubated in the same conditions*

	Thyroid tissue weight mg	Total cpm ^{125}I in the tissue	^{125}I tissue/ medium ratio	of ^{125}I in phosphate[a] extract	of ^{125}I in extract that was not dialysable[b]
Case 1	4 3	7 2 × 10^{3}	2 3	96	0
Case 2 while on thyroxine 0 15 mg/d	3 1	2 1 × 10^{4}	0 8	90	0
After 5 days of TSH stimulation[c]	{1 0 {3 6	{2 6 × 10^{4} {1 7 × 10^{4}	{1 6 {0 6	{83 {92	{2 {2
Case 3	2 6	3 6 × 10^{3}	113	91	40
Control material fetal thyroid glands Crown rump length 6–12 cm $n = 11$ The range is given			2–28	80–95	5–10

[a] 0 02 M sodium phosphate 0 1 M KCl pH 7 4
[b] Dialysis against the phosphate buffer for 16+4 h at +4 C
[c] Two biopsies examined

a tall girl growing along the 99th percentile She had not noticed any tenderness in the neck nor any fever or fatigue At the physical examination there were no signs of puberty and she was euthyroid The skin had normal texture and temperature Finger tremor was lacking The Achilles tendon reflexes were normal Her thyroid gland was conspicuously and symmetrically enlarged The surface was smooth but the gland was distinctly firmer than normal The results of the laboratory tests of her thyroid function are given in Table 1

METHODS

Biopsies from the thyroid glands were obtained percutaneously by a rotating needle developed and applied to thyroid biopsies by Ljunggren & Radner (7) The biopsy was performed either in local (prilocain) or general anaesthesia depending on the age of the patient Two to 5 mg specimens of thyroid tissue were recovered Where indicated a fragment was immediately immersed in 10 % formaldehyde for pathological examination The remaining tissue was used for biochemical studies as described in a previous paper on fetal thyroid glands (15) In brief the tissue was incubated for 24 hours at 37 C in 2 ml of Eagle s medium without leucine containing 200 μCi of ^{3}H leucine spec act 15–20 Ci/mmole 1–10 μCi of carrier free Na^{125}I and 6 mg of bensylpenicillin The tissues were weighed and the ^{125}I radioactivity was measured in the tissue and the medium The ratio of the ^{125}I radioactivity per mg tissue/^{125}I radioactivity per μl medium was calculated The soluble proteins were extracted with a KCl phosphate buffer dialysed and separated by sucrose density gradient centrifugation Each fraction of the gradient was analysed for ^{3}H and ^{125}I radioactivities and absorbance at 210 nm after dilution 1 to 3 Relevant peak fractions were analysed by immunochemical precipitation with rabbit antiserum against human 19 S thyroglobulin as described earlier (14)

Analytical methods

The methods used were serum PBI (1) serum TSH by radioimmunoassay (13) radioiodine uptake employing oral ^{131}I (3) Perchlorate discharge test was performed 70–75 min after i v administration of ^{132}I (1 μCi in Case 1 100 μCi in Case 3) according to Nilsson (11) In Case 1 100 mg of NaHClO and in Case 3 500 mg of NaHClO were administered orally

Passive haemaeglutination tests were performed to titrate antibodies to thyroglobulin indirect immunofluorescence and complement fixation techniques were used for detection of antibodies to thyroid cytoplasmic antigen as described (5)

RESULTS

The results of the thyroid function tests are summarized in Table 1 Case 1 was hypothyroid as judged by a low PBI in the neonatal period and an elevated serum TSH which decreased during therapy with thyroxine ^{131}I uptake in vivo was rapid in the thyroid region Perchlorate rapidly discharged 30 % of the accumulated radioiodine indicating a defect in the organification of iodine

In Case 2 the PBI was low at age 16 months ^{131}I was accumulated in the thyroid region and

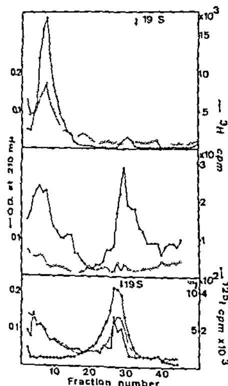


Fig 1 The sucrose density gradient centrifugation pattern of the crude soluble extracts from the thyroid biopsies of Case 1 (*top*) Case 2 first biopsy before TSH therapy (*middle*) and Case 3 (*bottom*) The glands were incubated in the presence of 200 μCi of ³H leucine and 1–10 μCi of Na¹²⁵I Sucrose gradient 5–20% Centrifugation for 225 min at 200 000 *g* No ¹²⁵I radioactivity was detected in Cases 1 and 2 19 S denotes the location of the peak of absorbance at 280 nm of purified adult human thyroglobulin run in the same rotor Sedimentation is from left to right

could partially be discharged by perchlorate This child was clinically possibly hypothyroid The perchlorate test suggested a defect in the organification of iodine although the technique used only allowed semiquantitative evaluation

Case 3 was not clinically hypothyroid The laboratory data showed a normal PBI but an elevated TSH level in the serum The radioiodine uptake was in the upper normal region The iodine excretion in the urine was definitely below the normal limit. Antibodies against thyroglobulin and thyroid cytoplasm antigens were present in the serum although cytological aspiration biopsy did not show signs of thyroiditis This child could still compensate her failing thyroid gland by an increased TSH stimulation

Biochemical in vitro studies

In Table 2 is shown the ¹²⁵I tissue/medium ratio in the biopsies from the 3 patients and from human fetal thyroid glands incubated under the same conditions Case 1 accumulates radioiodine most of which was found in the soluble extract although as shown in the table no ¹²⁵I radioactivity is left in the non dialysable fraction This indicated that the gland concentrated radioiodide but did not organify radioiodine in vitro In Case 2 the first biopsy performed while she was treated with thyroxine did not accumulate iodine After treatment with 1 U of thyrotrophin (Actyron® Ferring Malmo Sweden) per day for 6 days two biopsies were obtained At this time there was a slight accumulation of ¹²⁵I in one of the specimens Some ¹²⁵I radioactivity remained in the non-dialysable fraction although it was too low to be traced in the subsequent analysis of the soluble proteins by ultracentrifugation on a sucrose density gradient

In contrast to the first two goitres the thyroid specimen from Case 3 showed a higher ¹²⁵I tissue/medium ratio than the fetal thyroid glands The non-dialysable portion of the radioactivity was also augmented The data do not permit conclusions on the relative proportions between an increase in the concentration and organification of radioiodine as the ¹²⁵I tissue/medium ratio in the experiment includes both processes

In Fig 1 is shown the result of the sucrose density gradient centrifugation of the soluble proteins from the incubated thyroid biopsies

In Cases 1 and 2 there was no radioiodine detectable in any fraction The protein pattern as judged by absorbance at 210 nm showed a low molecular peak but no 17–19 S proteins ³H label was present in the slow sedimenting proteins In both patients there was also a ³H labelled peak with a sedimentation of 17–19 S as compared with human adult 19 S thyroglobulin run in the same rotor In the

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No radioiodine was detectable in the thyroglobulin present as identified by sucrose density gradient centrifugation ^{3}H leucine incorporation and immunochemical precipitation of the ^{3}H activity with antithyroglobulin serum The estimated sedimentation coefficient of 17–19 S confirms the data on the sedimentation rate of uniodinated thyroglobulin (12) Quantification of the amount of this protein was not possible with the method used

No radioiodine was present in any soluble or particle bound proteins Nor was there any detectable incorporation of tritiated leucine into abnormal proteins as compared to the pattern observed in the fetal thyroid gland (15) The data agree with those of Milutinovic et al (9) and Mouriz et al (10) who did not find any abnormal iodoproteins in one respective in 2 patients with nodular goitre organification defect and deafness (the so called Pendred s Syndrome) Medeiros Neto et al (8) found a particulate thyroid iodoprotein after in vitro labelling in one patient with nodular goitre organification defect and deafness The varying results may be explained by different pathogenesis in each case The possible biochemical heterogeneity of the individual nodular goitres that were examined by these authors makes interpretations very hazardous

The results obtained in Case 2 were disappointing The biopsies were performed while substitution therapy was given The thyroid gland was inactive as judged by the histological picture and the poor in vitro incorporation of ^{1} I and ^{3}H leucine into the thyroid proteins A 6 day course of TSH therapy only slightly augmented the in vitro uptake of radioiodide but failed to increase the ^{3}H leucine incorporation We did not feel justified to stop the substitution therapy for a short period of time in this patient at age 4 years because of the slight possibility of causing brain damage In a 7 year-old patient with congenital goitre and hypothyroidism successfully treated with thyroxine since age 6 months therapy was withheld for 5 weeks until overt hypothyroidism increased TSH levels and the reappearance of a minute goitre was noted Biopsies on this patient gave such a low yield that the biochemical analysis was unrewarding (unpublished data) The experience obtained from these 2 patients makes me reluctant to continue refined biochemical thyroid studies on goitres in patients who have been subjected to treatment with thyroxine

In Case 3 the in vitro biochemical findings indicated an augmented uptake and utilization of radioiodine in the thyroid tissue as judged by the increased tissue/medium ^{1} I ratio and the high percentage of the isotope found in the non-dialysable protein bound fraction of the soluble thyroid extract ^{1} I and ^{3}H were incorporated into thyroglobulin as identified by sucrose density gradient centrifugation No abnormal iodinated or uniodinated protein was detected The pathogenesis of the rapidly increasing goitre in this 9 year old girl seemed to be complex She had an iodine deficiency as judged by her low urinary excretion of iodine Genetically there was a family history of goitre Furthermore the tests of autoimmune reactions were inconsistent and did not exclude a thyroiditis The biochemical findings in vitro did not contradict the diagnosis of iodine deficiency

SUMMARY

Three patients aged 18 days 4 and 9 years respectively with childhood goitre were studied by labelling in vitro with ^{1} I and ^{3}H leucine of microbiopsies from the thyroid gland The soluble thyroid proteins were isolated by means of sucrose density gradient centrifugation

The first patient was clinically hypothyroid and showed a positive perchlorate discharge test His thyroid tissue did accumulate iodide but incorpated only ^{3}H into uniodinated thyroglobulin identified by a sedimentation coefficient of 17–19 S and reactivity to antiserum against human adult thyroglobulin The clinical and biochemical findings suggest that there was a defect in the organification of iodine with a qualitatively unaltered synthesis of the protein part of thyroglobulin

Table 3 *Immunochemical precipitation of ³H and/or ¹²⁵I labelled human thyroglobulin from Case 1 and Case 3 with rabbit antiserum against human adult thyroglobulin*

Sucrose gradient fractions from the 17–19 S peak were dialysed against the standard buffer To one aliquot of each sample no antiserum was added (Assay No 1) To another aliquot was added 20 μl of anti human adult thyroglobulin serum (Assay No 2) Inhibition of ^{125}I precipitation was measured by using another aliquot containing in addition 2 mg of unlabelled purified adult thyroglobulin (Assay No 3) After incubation at 37 C for 30 min and at 4 C for 24 h the mixture was centrifuged at 10 000 g for 30 min The supernatants were measured for either ^{3}H radioactivity in a liquid scintillation counter or ^{125}I radioactivity in a well scintillation counter (14) As control was used human fetal thyroglobulin obtained from *in vitro* incubation of ^{3}H leucine with the thyroid gland from a fetus of crown rump length 9 2 cm

Subject	Total radioactivity per assay: cpm ^{125}I	Total radioactivity per assay: dpm ^{3}H	Assay No 1 Non specific precipitation of radioactivity	Assay No 2 Radioactivity precipitated by antithyroglobulin serum	Assay No 3 Radioactivity precipitated in the presence of unlabelled thyroglobulin
Case 1		$2\ 5 \times 10^{3}$	10	73	11
Case 2	$1\ 8 \times 10^{3\,a}$		1–6	97–100	0
Control		$2\ 1 \times 10^{4}$	13	70	26

[a] Two samples were tested

biopsy obtained after 5 days of therapy with TSH to Case 2 the ^{3}H radioactivity did not increase in the 17–19 S region

In Case 3 a protein peak was present in the 17–19 S region labelled with ^{125}I and ^{3}H The slow sedimenting protein peak was labelled with ^{3}H There were no ^{125}I and ^{3}H in any unusual or unexpected area of the gradients as compared with normal thyroid proteins in the human fetus The peak fraction of the 17–19 S ^{3}H activity in Case 1 and the 17–19 S ^{125}I peak fraction in Case 3 were used for immunoprecipitation with antithyroglobulin serum Table 3 Both peaks contained radioactivity that was mainly precipitable with antithyroglobulin serum The precipitate was not due to unspecific absorption of the radioactivity to the precipitate complex since it could be inhibited by the addition of an excess of unlabelled human 19 S thyroglobulin

DISCUSSION

This preliminary report presents data on the soluble thyroid proteins in three patients with diffuse goitres during childhood The biochemical assays were made on microspecimens obtained by rotating biopsies according to the technique developed by Ljunggren & Radner (7) This technique offers distinct advantages as compared with biopsies obtained at open surgery or biopsies taken by the Silverman technique It is less traumatic and may be performed on an outpatient basis The simplicity of the procedure facilitates biochemical studies on infants with a goitre before the thyroid glands has become nodular due to prolonged TSH stimulation or substitution therapy has been instituted

The in vitro technique circumvents the ethical problems involved in the use of radioiodine in childhood (19) The limitations of this biopsy technique depend on the small size of the samples obtained—2 to 10 mg Analysis of enzymes involved in the synthesis of the thyroid hormones may not be performed Likewise determination of the amount of different cellular constituents such as microsomes or chemical determination of iodine is not practicable at present The rapid development of different micromethods may very well extend the applicability of the method It should be pointed out that studies on biopsy specimens may not be representative for other portions of the thyroid gland (17)

Case 1 had a goitre hypothyroidism and a positive perchlorate discharge test Clinically the disease could be classified as an organification defect The in vitro results suggested that the thyroid gland did concentrate iodide

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THE INCIDENCE AND GENETICS OF METACHROMATIC LEUCODYSTROPHY IN NORTHERN SWEDEN

K H GUSTAVSON and BENGT HAGBERG

From the Department of Paediatrics University Hospital Uppsala Sweden

It has been possible for about 10 years to diagnose m tachromatic leucodystrophy (MLD) or sulphatide lipidosis with c rtainty during life by means of urinary tests (1 2 6 7) or biop y examination of p ripheral nerve speci mens (8 18) With th introduction of tests on the activity of the component aryl sulfatase A of th enzyme c rebroside sulfatase in the urine (3) it has even become possible to reveal the disease in the neonatal period in a pre clinical stage (5) Very exact enzymatic tests on leucocyte concentrates (14) and cultured fibroblasts (12) have also become available during recent years Thus th necessary diag nostic tools now exist for mapping down the frequency and the mode of inh ritance of this disord r

Th aim of this investigation was 1) to find out the incid nce of MLD in the two most orthern regions for health and sick care of Sweden (Umeå and Uppsala regions consisting of the counties of Norrbotten Vasterbotten Vasternorrland Jamtland Kopparberg Gavle borg Vastmanland and Uppsala see Fig 1) with a total population of about 2 million people and 2) to perform g netic and genealog ic studies on the clinical material collected The study was facilitated by the fact that one of th authors (B H) served as the consultant child neurologist for the area in question during th period of the investigation i e 1956–1967 and consequently saw and investigated person ally all progressive neurological cases with any suspicion of this disease

MATERIAL

All children from the Umeå and Uppsala regions for health and sick care who during the period 1956–1967 presented symptoms characteristic or suggestive of MLD were investigated clinically by one of the authors (B H) During this period a definite diag nosis of late infantile MLD was established on clini cal histological and specific laboratory evidence in 11 children and another child a boy was found to have the juvenile form of MLD

Family data were collected from hospital records and from interviews with the parents Genealogic data on the patients and their relatives were col lected from the parish records and county archives In this way information was obtained on 2 additional children with a definite diagnosis of late infantile MLD born in 1953 and 1954 and each belonging to a sibship in the original group Investigation also revealed a boy with juvenile MLD born in 1945 who was a brother of the above mentioned child with the juvenile type of MLD Our total series of MLD thus consisted of 13 cases with the late infantile form from 11 sibships (Table 1) and 2 sibs with the juvenile form of MLD The 2 brothers with juvenile MLD were the 1st and 5th of 5 siblings The parents were healthy and no other cases of MLD were found among the relatives The youngest of the two affected brothers L K born February 2nd 1960 was 6 / years o age on manifestation of symptoms The clinical picture combined with patho gnomonic histochemical changes in the sural nerve biopsy a very low activity of urinary aryl-sulfatase A and the specific dihexose sulfatide spot in the urine proved the diagnosis of MLD The boy is still alive at 10 years of age but is in a poor general condition The age at manifestation of symptoms in the elder brother K A K born July 15 1945 was 5 / years.

The second patient was on substitution therapy because of hypothyroidism at the time of the study The use of similar methods after 1 week of exogenous TSH stimulation did not give any further information regarding the pathogenesis of the goitre

The third patient was euthyroid, compensating for iodine deficiency with increased secretion of TSH The biochemical studies revealed an increased in vitro accumulation of radioiodine and a normal synthesis of iodinated thyroglobulin as compared to the human fetal thyroid gland

No abnormal iodoprotein was detected in any of the patients

ACKNOWLEDGEMENTS

Professor A Fagræus M D, Stockholm, made the immunological assays of the serum samples I am indebted to Dr J G Ljunggren for placing the instrument for thyroid biopsy at my disposal

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Table 1. Family data on the patients with late infantile metachromatic leucodystrophy
M = male. F = female.

Family number	Number of children		Order in sibship	Sex of affected children	Age at manifestation of symptoms (months)
	Total	Affected			
1	1	1	1/1	F	15
2	1	1	1/1	F	18
3	2	2	1/1 2/2	F F	24 24
4	2	1	2/2	M	15
5	6	1	4/6	M	18
6	4	2	2/4 4/4	M M	24 15
7	3	1	3/3	M	9
8	2	1	2/2	M	15
9	2	1	2/2	M	9
10	1	1	1/1	M	15
11	3	1	3/3	F	18
Total	27	13			

He died at 11 years of age. The clinical picture and course of his disease will be described in detail in a further publication (11). Both cases fall within the age criteria of Moser & Lees (13) for juvenile MLD.

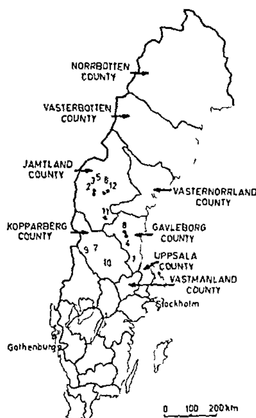


Fig. 1. Map of Sweden. Birth places of children with MLD in the Uppsala and Umeå medical regions are indicated by filled circles. The numbers indicate family numbers referred to in the text.

EPIDEMIOLOGY

Age at manifestation and incidence. For the 11 proband cases with late infantile MLD the mean age at manifestation of the disease was 14 months, with a range of 9 months to 2 years. Thus the cases of late infantile MLD born in the Umeå and Uppsala regions for health and sick care during the period 1955–1965 should be almost fully covered by the present material. During this period 8 cases of late infantile MLD were born. The number of births in the Umeå and Uppsala regions during the period 1955–1965 was 316 786. The birth incidence rate for late infantile MLD in that population can thus be calculated to be about 1 per 40 000. Only one case of juvenile MLD was born in the Umeå and Uppsala regions during the same period.

Geographical distribution. When the 15 patients in our total material were grouped according to the county in which they were born it was noted that all were born in the southern part of the investigated area comprising the counties of Jamtland, Kopparberg and Gavleborg (Fig. 1). These counties all border on each other. Six of the 13 children with late infantile MLD and the two sibs with juvenile MLD were born in a fairly restricted area in the central part of Jamtland county, where geographical population isolates are common.

GENEALOGY

The genealogic data of the patients and their parents were collected from parish register offices and county archives Genealogic data were obtained for at least 7 generations back for 6 of the 12 families and for more than 5 generations back for the other 6

A Late infantile MLD A consanguineous marriage was verified between the parents of each of three probands with late infantile MLD viz. family 5 (Fig 2) family 10 (Fig 3) and family 11 (Fig. 4) None of these were first cousin marriages In the other 8 families with late infantile MLD no indication of consanguinity between the parents was discovered

The ancestries of families 2 3 5 and 6 could be traced back to the years 1690–1730 and to the same central part of Jamtland county as where the probands were born The ancestry of family 1 was traced back to the year 1730 and to the same village in Gavleborg county as where the proband was born The ancestors of family 4 were traced back to the year 1700 and to the same village in Gavleborg county as where the proband was born

The ancestors of the father of the proband in family 8—from Gavleborg county—were traced back to 1710 and came from the county of Jamtland

The ancestors of family 10 from Kopparberg county were traced back to the beginning of the 18th century and to the same village as where the parents of the proband were born (Fig. 3)

The father of the proband in family 7—Kopparberg county—had common ancestry with the mother of the proband in family 9

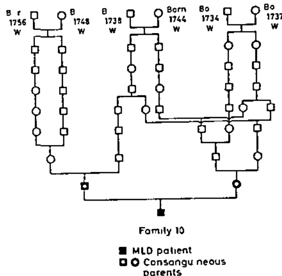


Fig 3 Pedigree of family 10 The birth county is indicated by a letter W denotes the county of Kopparberg

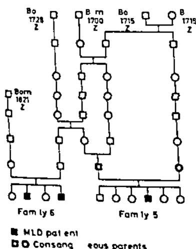


Fig 2 Pedigrees of families 5 and 6 The birth county is indicated by a letter Z denotes the county of Jamtland

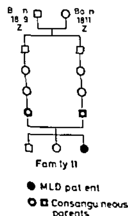


Fig 4 Pedigree of family 11 The birth county is indicated by a letter Z denotes the county of Jamtland

Table 1. *Family data on the patients with late infantile metachromatic leucodystrophy*
M = male, F = female

Family number	Number of children: Total	Affected	Order in sibship	Sex of affected children	Age at manifestation of symptoms (months)
1	1	1	1/1	F	15
2	1	1	1/1	F	18
3	2	2	1/1 2/2	F F	24 24
4	2	1	2/2	M	15
5	6	1	4/6	M	18
6	4	2	2/4 4/4	M M	24 15
7	3	1	3/3	M	9
8	2	1	2/2	M	15
9	2	1	2/2	M	9
10	1	1	1/1	M	15
11	3	1	3/3	F	18
Total	27	13			

He died at 11 years of age. The clinical picture and course of his disease will be described in detail in a further publication (11). Both cases fall within the age criteria of Moser & Lees (13) for juvenile MLD.

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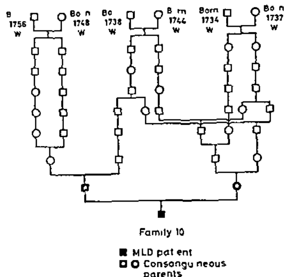


Fig 3 Pedigree of family 10 The birth county is indicated by a letter W denotes the county of Kopparberg

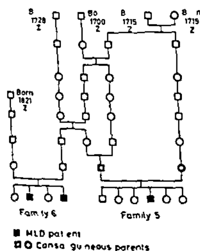


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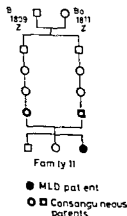


Fig 4 Pedigree of family 11 The birth county is indicated by a letter Z denotes the county of Jamtland

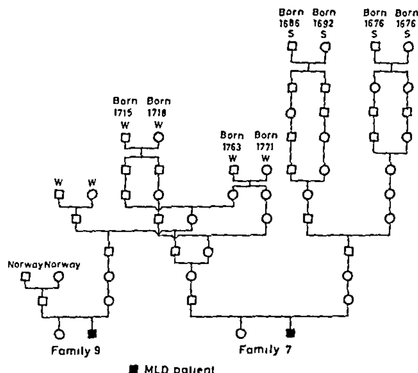


Fig 5 Pedigree of families 7 and 9 Birth counties are indicated by letters W denotes the county of Kopparberg S denotes the county of Varmland

from the same county (Fig 5) Blood relationship was also found between family 5 and 6 from the central part of Jamtland county (Fig 2)

B Juvenile MLD The ancestry of family 12 with juvenile MLD could be traced back 6 generations and to the same village in the central part of Jamtland county as where the pro band was born No indication of consaguinity was discovered between the parents of the affected male siblings and no common ancestor with any of the families with infantile MLD was traced

GENETIC ANALYSIS OF FAMILY DATA

The present data refer to 22 parents (11 couples) with a total of 27 children 13 of whom (8 boys and 5 girls) were affected with late infantile MLD (see Table 1) The parents were all healthy and no indication of late infantile MLD was found in at least three generations preceding that of the affected children No association between late infantile MLD and birth rank was found

As it is most probable that all cases of late infantile MLD born in the Umeå and Uppsala regions during the period of 1956–1967 were discovered and since the affected siblings were traced independently of the affected proband we applied Weinberg s proband method for the genetic analysis (16) This method gives the result $p = 0\ 20$ which is close to what should be expected from a simple recessive type of inheritance (0 25)

DISCUSSION

No incidence studies on MLD appear to have been reported in literature The present investigation has shown that the birth incidence rate of late infantile MLD in the northern half of Sweden is about 1 per 40 000 The analysis of the birth places of the patients revealed, however a geographical cluster in the central part of Jamtland county where population isolates are common It is of interest to compare these findings with the occurrence of infantile globoid cell leucodystrophy in Sweden This type of leucodystrophy which has about the same birth incidence rate as late infantile MLD, is however diffusely spread over the whole country (10)

The present study confirmed that the juvenile

form of MLD is much more rare than the infantile form

Except for a consecutive series of late infantile MLD investigated by Schutta et al (17) only case reports have been published (see Pratt 1967) The series of Schutta et al (17) consisted of twelve sibships and there was a total of eighteen affected and twenty unaffected children The analysis of this material was suggestive of autosomal recessive inheritance of late infantile MLD though no parental consanguinity was found The extensive pedigree study with enzyme determinations on heterozygotes by Bass et al (4) of a large family with four patients suffering from late infantile MLD was highly suggestive of autosomal recessive inheritance

The results of the present investigation also strongly support an autosomal recessive inheritance in late infantile MLD affected relatives were confined to sibships the parents were normal the sex ratio of affected cases did not differ significantly from unity parental consanguinity was found in three of the eleven families the proportion of affected children in the families corrected according to Weinberg s proband method agrees fairly well with what is expected in autosomal recessive transmission

The concentration of the birth places and ancestry of 6 of the 13 children with late infantile MLD to a certain restricted isolated area of Jamtland county is an indication that the gen could have had its origin in a single gene mutation a long time ago The genealogic analysis has not been able to prove this but one indication is the blood relationship found between two of the families in the isolated district in Jamtland and between two of the families from the county of Kopparberg as well as the ancestor line from the same district in Jamtland in family 8 from Gavleborg county

The juvenile group of MLD seems to be less homogeneous both clinically genetically and with regard to age at onset than the late infantile cases In a few families there appears to be a dominant or X linked recessive inheritance but in the majority of published cases autosomal recessive inheritance is suggested (15)

In the family in our series with two brothers affected with juvenile MLD a recessive mode of inheritance either autosomal or X linked appears likely It has been suggested that the juvenile type of MLD with onset between 4 and 10 years of age represents cases of the late infantile form with a late onset The parents of the two brothers with juvenile MLD in our series were born in the same isolated part of Jamtland county as 6 of our cases with the late infantile form of the disease This family lived in the same little Jamtland village with a population of 267 as family 5 with late infantile MLD However the genealogic analysis with penetration of 6 generations showed no relationship between the family with juvenile MLD and the families with the infantile form

SUMMARY

The purpose of this study was to investigate the incidence and genetics of metachromatic leucodystrophy (MLD) A series of 13 cases of late infantile MLD and 2 cases of juvenile MLD was collected from the northern part of Sweden and studied together with their relatives The series was considered to be adequately representative of the true occurrence of MLD during the period 1955–1965 The incidence rate for late infantile MLD was estimated to be about 1 per 40 000 The MLD patients were highly concentrated to the south west part of Norrland From the family analysis it was concluded that late infantile MLD is most probably transmitted by an autosomal recessive gene

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SHORT COMMUNICATION

TRISOMY 18 IN GREECE

Seven Cases of Pure Trisomy 18 and One with a D/G Translocation

O M CHRYSOSTOMIDOU E CASLARIS D ALEXIOU and C S BARTSOCAS

From the Institute of Child Health Athens and the Neonatal Unit State and University "Alexandra" Maternity Hospital Athens Greece

Trisomy 18 syndrome is now a well defined entity and more than 200 cases have been recorded since the first descriptions by Edwards et al (3) and Patau et al (8) in 1960 The pathology of this multiple anomaly syndrome is attributed to the presence of the extra chromosome 18 but its etiology remains unknown as it is for most other chromosomal disorders

Warkany et al (10) recently stressed the need for detailed clinical and pathological reporting of cases published because of chromosomal aberrations The purpose of this article is to present data on 7 newborn Greek infants with this syndrome These infants were studied at the Cytogenetic Centre of the Institute of Child Health Three of them were discovered during a screening for genetic disorders of all newborns delivered at the "Alexandra" Maternity Hospital between 1966 and 1968 in a joint study of the Institute of Child Health and the Neonatal Unit of the "Alexandra" Maternity Hospital (7)

These authors reported their data on a study of 10 412 liveborn Greek infants for sex chromatin anomalies Karyotypes performed in all newborns with multiple congenital abnormalities revealed 3 cases of trisomy 18 an incidence of 0 03% The incidence therefore of the trisomy 18 syndrome in our area does not differ significantly from the ratio of 1 4 500 births observed elsewhere (2 5)

Clinical and autopsy information on all patients is summarized in Tables 1 and 2 All patients except for case 3 had karyotypes with 47 chromosomes the extra one being identified as of the E group The parents karyotypes were found to be normal Case 3 however had 46 chromosomes with an extra 18 but with a balanced D/G translocation Her mother had normal chromosomes but her father s chromosome study showed a balanced D/G translocation Blood karyotype data on patients and their parents are summarized in Table 3

The preponderance of females in our cases (6 1) seems to be in accordance with the increased ratio of affected females to males

Table 1 *Sex parental age weeks of gestation and birth weight of patients with trisomy 18*

Case no	Sex	Maternal age at birth	Paternal age at birth	Weeks of gestation	Birth weight (grams)
I	F	43	33	?	1 600
II	M	37	40	43	2 030
III	F	23	30	40	2 400
IV	F	24	42	42	2 020
V	F	33	36	42	3 150
VI	F	25	31	43	1 980
VII	F	41	32	38	1 900

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The variability of the clinical picture in trisomy 18 can be compared with other syndromes of multiple congenital anomalies manifesting themselves as spectra of congenital malformations This makes necessary confirmation of the diagnosis on etiological grounds Nonetheless it is important to collect data from large series as a possible break through to the etiology of the syndrome

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Table 2 *Anomalies observed on 7 cases of trisomy 18*

Anomaly	Cases I	II	III	IV	V	VI	VII
Single umbilical artery	–	–	+	–	?	–	?
Apparent mental deficiency	+	+	+	+	+	+	+
Prominent occiput	+	+	+	+	+	–	+
Low set malformed ears	+	+	+	+	+	+	+
Eyes ptosis & inner epic folds	–	–	–	–	–	–	–
Small palpebral fissures	+	+	+	+	+	+	+
Corneal opacities	–	–	–	–	–	–	–
Micrognathia	+	+	+	+	+	+	+
Narrow palatal arch	+	+	+	+	+	+	+
Small oral opening	+	+	+	+	+	+	+
Cleft lip or palate	–	–	–	–	–	–	–
Fingers flexed	+	+	+	+	+	+	+
Index overlaps 3rd/5th over 4th	+	+	+	+	+	–	+
6 or more low arches	?	?	+	+	?	+	+
Simian crease	–	+	–	+	?	+	+
Single crease 5th finger	–	+	–	+	?	–	+
Hypoplastic fingernails	–	–	–	–	–	–	–
Hypoplastic thumb	–	–	+	–	–	–	–
Short hallux	+	+	+	+	+	+	+
Rockerbottom feet	+	+	+	+	+	+	+
Equinovarus	+	+	+	+	+	–	–
Syndactyly 2nd–3rd toe	–	–	+	–	–	–	–
Short sternum	+	+	+	+	–	–	+
Wide spaced nipples	+	+	+	+	+	+	+
Small pelvis	+	–	+	+	+	+	+
Limited hip abduction	+	–	+	+	+	+	+
Anal anomalies	–	–	–	–	–	–	–
Cryptorchidism		+					
Prominent clitoris	+		–	+	–	–	–
Gonadal dysgenesis	–		–	–	–	+	?
Abnormal uterus	–		–	–	–	–	?
VSD	–	+	+	+	–	+	?
ASD	–	–	–	+	–	–	?
PDA	–	–	+	+	+	–	?
Double ureter	–	+	–	–	–	–	?
Meckel s diverticulum	–	–	–	+	–	–	?
Inguinal hernia	–	+	–	–	–	–	–
Abnormal lobation of liver	+	+	+	–	–	+	?
Hypoplastic gallbladder	–	–	–	–	–	+	?
Kernicterus	–	–	–	–	+	–	–

found by Ferguson Smith (4) and Weber (11) Although these authors explain this ratio by proposing a greater mortality of males during the period shortly after birth, we believe that this explanation should be valid for the intra-uterine mortality of defective males, rather than in the neonatal period This of course

Table 3 *Blood cytogenetic data (I) (Method of Moorhead et al (6))*

Case no	Patient	Father	Mother
I	47 XX 18+	46 XY	46 XX
II	47 XY 18+	46 XY	46 XX
III	46 XX 18+ D G t (DqGq)+pat	45 XY D G t (DqGq)+	46 XX
IV	47 XX 18+	46 XY	46,XX
V	47 XX 18+	46 XY	46 XX
VI	47 XX 18+	46 XY	46 XX
VII	47 XX 18+	46 XY	46 XX

requires confirmation by collection of large abortion material statistics

The effect of trisomy 18 is almost always lethal in early infancy, although a small number of individuals survived beyond this age All, but one, of our cases died within the first month of life

The only factor possibly related to the etiology of this syndrome is a maternal age effect 4 of the 7 mothers of our patients were 33 years or older These figures agree with Smith s (9), who estimated that 52 5% of the mothers of trisomy 18 patients were over 35 years old This suggests as a causative factor of the syndrome, either a defective ovum, because of some pre-conceptive ill effect or just non disjunction due to an aging ovum As for case 3, where a balanced translocation existed in the father, it would be interesting to know whether this chromosome anomaly could have caused non disjunction on another autosome

Except for the mother of case 6 who since the twentieth week of gestation took Diphenal[1] for control of epileptic seizures no drugs were taken, no X rays were performed and no infections during pregnancy were reported Most of our patients were born after a gestation of more than 40 weeks with a low birth weight (less than 2 500 g) The average birth weight of our patients was 2 154 g which agrees with the average weight (2 183 g) of 90 cases of trisomy 18 analysed by Warkany et al (10)

[1] Combination of Sodium Diphenylhydantoinate and Phenylethylbarbituric acid

The variability of the clinical picture in trisomy 18 can be compared with other syndromes of multiple congenital anomalies manifesting themselves as spectra of congenital malformations This makes necessary confirmation of the diagnosis on etiological grounds Nonetheless it is important to collect data from large series as a possible breakthrough to the etiology of the syndrome

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Key words Trisomy 18 D/G translocation double aneuploidy

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CASE REPORT

A VARIANT FORM OF BRANCHED CHAIN KETO ACIDURIA

J L VAN DER HORST and S K WADMAN

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Branched chain keto aciduria (BCKA) also called maple syrup urine disease is a neurometabolic disorder caused by inability to metabolize the branched-chain amino acids leucine isoleucine and valine, which with their corresponding keto and hydroxy acids, accumulate in all the body fluids The enzyme defect is localised at the level of the oxidative decarboxylation of these keto acids The disease may take an acute form leading if not promptly treated, to rapid and irreversible neurologic damage There is also an intermittent form producing symptoms at a later age and only under special provocative conditions such as fever infections vaccinations and excessive protein intake (1 4 7, 9 11) Keto acidotic attacks appear suddenly and can be rapidly fatal unless diagnosed correctly and given urgent temporary dietary treatment Patients with this intermittent form of BCKA show a normal mental development and the neurological abnormalities are present only during attacks

Recently, 2 cases have been described with yet another clinical picture Schulman et al (13) reported a case of permanent BCKA in a moderately retarded girl of 1 ¹/ years showing no neurological abnormalities and no attacks of severe acidosis Gerritsen et al (6) during routine screening discovered a moderately retarded girl of 18 years with severe BCKA and slight hyperamino aciduria who also had no neurological symptoms

In the case reported here a boy was admitted to hospital at the age of 7 months with signs of BCKA but with no history of previous illness At the time his disease was recognized he showed a severe mental retardation The institution of dietary treatment resulted in a favorable clinical course so that at the age of 3 years the boy s mental development corresponded with his age

CASE HISTORY

W was first admitted to the hospital for vomiting at the age of 7 months 10 days after a smallpox vaccination The past history was uneventful

Examination revealed a pale semicomatose infant in a dehydrated state His weight was 6 280 g He had a metabolic acidosis the pH of the blood was 7 17 and the standard bicarbonate concentration 10 6 mEq/l The CSF contained 9/3 lymphocytes/mm and 31 mg/100 ml of protein The EEG showed a slight disturbance of the left hemisphere Intravenous infusions of sodium bicarbonate were started and the metabolic acidosis was corrected in 60 hours The patient gradually recovered and was discharged well the BCKA syndrome remained unrecognized

The boy was readmitted to the hospital at 9 months for a respiratory infection He was pyrexial and his sedimentation rate was 35 mm The total plasma protein concentration was normal The boy s blood glucose was 49 mg/100 ml The CSF now contained /ₛ lymphocytes/mm and 17 mg/100 ml of protein During his admission at the age of 11 months a transient ataxia was noticed

Table 1 *Urinary amino acids (mg per g creatinine) of patient W during admission*

Age (months)	9	10	11	17	Treatment started 18	Normals $n = 10$
Valine	71	67	117	58	56	
Allo-isoleucine	10	18	11	20	—	—
Isoleucine	31	90	58	33	30	<10
Leucine	31	114	212	62	40	<41

A two-dimensional paper chromatogram showed increased urinary branched chain amino acids. Their corresponding keto acids were also present. These abnormalities highly suspect for BCKA became more pronounced in further examinations. A peculiar odor of the urine was also noticed when the patient had a gastro-enteritis.

Table 1 shows the urinary branched chain amino acids analysed by quantitative column chromatography according to the Technicon 22 hour method. The excretions were moderately increased or still normal. The increase is less impressive than is usual in patients with the classical form of BCKA. Allo-isoleucine, a characteristic product in this disorder, occurred in all samples until treatment was started. In the plasma only branched chain amino acids were increased (Table 2) suggesting that some form of BCKA might be present.

At the age of 17 months, before dietary treatment was started, the patient had a severe ataxia. The EEG was abnormal (paroxysmal and bilaterally synchronous delta waves and once a sign of a more local left fronto-temporal disorder). At this time the boy was examined by a psychologist, who reported severe developmental retardation (Table 3).

Dietary treatment. We decided to treat this patient with a diet low in branched-chain amino acids. According to the directions of Ireland (8) one of the

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nitrogen containing components of this diet consisted of 3 parts of gelatin and 1 part of albumaid (mixture 1). The other nitrogen-containing component (mixture 2) contained amino acids only (Table 4). The complete composition of the diet used when the treatment was started is shown in Table 5.

At first the administration of the diet raised problems because the patient partly refused mixtures 1 and 2. Gradually these difficulties disappeared. Three weeks after starting dietary treatment, when the patient was 18 months old, analysis of the plasma and urinary amino acids showed a striking improvement (Tables 1 and 2). However, the plasma concentrations of methionine, tyrosine and phenylalanine were on the low side. For this reason 400 mg phenylalanine and 800 mg methionine were added to the daily ration. A month later valine was normal but leucine and isoleucine were somewhat low (Table 2). Methionine and tyrosine were to high. Three weeks later the concentrations of methionine and tyrosine became low. A further addition of 100 mg of methionine and also 900 mg of tyrosine was made to the daily diet. The daily intake of mixture 2 was also increased to 26.5 g. At the age of 21 months increased plasma concentrations of leucine, valine and isoleucine were again found and allo-isoleucine reappeared. This was probably due to excessive protein catabolism since the patient had a respiratory infection and vomited at this time. The plasma methionine level was low but the tyrosine and phenylalanine levels were in the normal range. Once again in

Table 2 *Plasma amino acids (mg per 100 ml) of the patient W*

Age	Before treatment 17 (mo)	After start of treatment 18 (mo)	19 (mo)	19½ (mo)	21 (mo)	23 (mo)	26 (mo)	31 (mo)	3 1/12 (y)	3 1/12 (y)	Normal (mean + 2 S.D.)
Valine	14.6	1.75	[illegible].18	2.06	3.64	4.65	2.56	2.40	[illegible].84	1.88	3.63
Allo-isoleucine	1.35	—	—	—	trace	trace	—	—	—	—	—
Isoleucine	6.4	0.59	0.18	0.53	1.66	1.63	0.76	0.95	0.94	0.91	1.16
Leucine	15.1	1.78	1.00	1.90	4.06	4.59	2.30	2.70	2.90	2.[illegible]8	2.79
Methionine	0.[illegible]8	0.16	3.25	0.15	0.17	0.48					0.49
Tyrosine	0.98	0.38	3.34	0.52	0.6[illegible]	1.01					1.4[illegible]
Phenylalanine	1.15	0.6[illegible]	1.46	0.77	0.83	1.44					1.28

creased plasma concentrations of branched chain amino acids were observed at the age of 23 months the patient had another 3 days' attack of vomiting and loss of weight caused by an infection No other symptoms were seen No further abnormalities have appeared up to the time of writing

Development after starting dietary therapy

Soon after dietary treatment was instituted the ataxia disappeared Two months later when the patient was 20 months old (Fig 1) the EEG was normal

The psychologist reported a favorable progress of motor function and social behavior At 28 months the psychological evaluation revealed that the retardation of the motor function was disappearing (Table 3) At 3 years the boy s mental development corresponded with his age His speech however was still retarded but this defect could not easily be evaluated as his brothers also had transient speech difficulties This defect may possibly have no direct connection with BCKA

The patient s physical development was satisfactory After his discharge from the hospital there was a satisfactory gain of height and body weight At the age of 3 years and 9 month his height was 101 cm (50th percentile) and his weight 16 3 kg (50th percentile) Occasional respiratory infections occurred and once he was readmitted for a gastro-enteritis However the clinical symptoms of BCKA did not reappear

Examination of the family

The patient W was the youngest of the 5 children of healthy parents A sister M (third child) died in coma at the age of 21 months She had a respiratory infection with vomiting and developed a metabolic acidosis which could not be corrected A peculiar odor of the urine was noticed Probably she had the same disease as her brother W The fourth child suffered from epilepsy but was otherwise healthy and was mentally normal as were the two eldest children

Heterozygotes can be detected by direct estimation of the decarboxylase activities in peripheral leucocytes using isotope techniques (3 4 7) Oral leucine loading tests may also be informative (10 15 16) The reliability of both methods has to be further evaluated and it is still uncertain to what extent the 2 methods agree The latter method may throw more light on the metabolic defect in the whole subject while the former is more closely connected with the typical enzyme system of a special cell

Leucine tolerance tests were performed on the present patient s family 150 mg of l leucine per kg being given by mouth As can be seen from Table 6 all members of the family tested had an abnormal curve according to the criteria of Woody & Harris (16) Presumably all are heterozygous carriers Leucine intolerance was more pronounced in the father than in the mother Similarly the abnormal gene seemed to be more active in the two brothers than in the patient s sister

The family s pedigree is shown in Fig 2 The female parent in the fourth remove from the father s side was a sister of the male parent in the fourth remove from the mother s side

It is also noteworthy that a sister of the patient s mother married a brother of the patient s father Their 7 children remarkably enough are all in good health and mentally normal

Table 3 *Psychological examination of W*

(1) *Tested with the Buhler-Hetzer infant test 1953*[a]

Age 1 5+17	Developmental quotient 0 80 Motor development low
Age 1, 9+1	Developmental quotient 0 83 *Motor and social improvement*
Age 2 4+21	Developmental quotient 0 81 Motor and social development approximately in accordance with age

(2) *Tested with the Stanford–Binet test* Dutch version described by L M Terman & M A Merrill Measuring Intelligence 1955 type L

Age 2 4	Mental age cannot be determined Fails on all verbal items of II and II 6 Can build and complete formboard
Age 3, 10	Mental age 3 6 *IQ 91* Fails on verbal items Drawing and building in accordance with age
Age 4 10	Mental age 4 11 *IQ 102* Retardation abolished No typical outlying values in profile

Table 4 *Composition of the protein and amino acid mixtures 1 and 2 used for treatment*

	Mixture 1 Albumaid Gelatin (mg/g)	Mixture 2 Amino acids (mg/g)
Alanine	65 0	—
Glycine	181 2	353
Proline	120 3	—
Glutamic acid	92 6	353
Aspartic acid	56 0	—
Serine	32 4	—
Threonine	20 4	20 6
Cystine	5 2	17 6
Methionine	8 5 (l)	41 2 (dl)
Tyrosine	8 2	61 8
Phenylalanine	21 7	41 2
Histidine	8 8	32 3
Arginine	66 1	—
Lysine	52 6	59
Tryptophan	17 0	20 6
Leucine	33 5	—
Isoleucine	18 0	—
Valine	27 1	—

Table 5 *Composition of the diet of patient W (11.3 kg) at start of therapy*

Leucine 90 mg/kg isoleucine 55 mg/kg valine 71 mg/kg Calories 1321

	Leucine (mg)	Isoleucine (mg)	Valine (mg)	Protein (g)	Fat (g)	Carbohydrate (g)
4 aminex biscuits	23	18	18	0.5	4	44
45 g margarine	—	—	—	—	37	—
40 g jam	—	—	—	—	—	24
2 fruits	—	—	—	—	—	20
50 g potatoes	60	55	50	1 0	—	10
60 g vegetables	56	44	36	1 0	—	2
50 g sugar	—	—	—	—	—	50
40 ml cream 40	70	79	46	1 0	16	—
74 g mixture 1	804	432	650	(22)	—	—
4 g mixture 2	—	—	—	(2.)	—	—
5 15 g mineral supplement (Ireland)[a]	—	—	—	—	—	—
Vitamins normal administration						

[a] 100 g mineral mixture contained 17 6 g Ca 1 3 g K 0 63 g Na 0 31 g Mg 0 14 g Fe 0 019 g Zn 0 015 g Cu 0 013 g Mn 1 2 mg I 0 3 mg Mo 0 2 mg Co 0 04 mg Al 71.2 g lactate 2 8 g Cl 0 97 g P 1 53 g SO_4

DISCUSSION

Our knowledge of the enzymes of the branched chain alpha keto acid dehydrogenase systems is still too incomplete for a quantitative interpretation to be made of the metabolic patterns in BCKA The accumulation of leucine and its metabolites is greater than that of other branched-chain compounds this has been interpreted as indicative of separate dehydrogenases However other factors must also be considered such as the inhibition of enzyme activity by related keto acids and the conduction of dehydrogenases by related amino acids Both factors have been studied extensively (14) In the rat two superimposable mechanisms seem to be operative in modulating the dehydrogenase activities adaptation to the dietary protein intake and a daily activation–deactivation cycle Bowden & Connelly (2) obtained evidence of the existence of a single enzyme complex for the oxidative decarboxylation of both alpha keto-isocaproic acid (derived from leucine) and alpha keto-beta methyl valeric acid (derived from isoleucine) and a separate enzyme complex for the oxidative decarboxyla



Fig 1 The patient at the age of 20 months

Table 6 *Leucine tolerance tests on patient's parents brothers and sister*

Plasma leucine in mg/100 ml after an oral load of 150 mg l leucine per kg bodyweight

	Fasting	1 h	2 h	3 h
Mother	2.7	9 4	12.5	10 9
Father	2.9	12 8	17 0	13 5
Sister aged 9 y	1 7	13 6	9 4	3 9
Brother aged 7 y	2.9	16 6	1_5	7 9
Brother aged 5 y	2.9	13 1	10 4	6 0
Max values in 5 normals Woody & Harris (16)	2.5	10	7 7	6.5
Normal child (analysed in author's laboratory)	1 40	6 83	2.83	2.32

tion of alpha keto isovaleric acid (derived from valine) in bovine liver Goedde et al (7) however, argued for the existence of 3 different decarboxylating enzymes for each of the branched-chain keto acids The experimental results of Wohlhueter & Harper (14) may be interpreted in 2 ways either a single enzyme complex is responsible for the oxidation of all three branched chain keto acids, or if there are multiple enzymes they are subject to a very exacting co ordination in their regulation

Whatever the detailed structure of the enzyme systems may be there is strong evidence that in classical BCKA the enzyme defect is complete or nearly complete whereas in the intermittent form and other variants decarboxylase activities are less severely reduced (3, 6 7 13)

Classical BCKA is transmitted as an autosomal recessive defect The decarboxylase activities in the leucocytes of both parents of patients with this classical form are reduced (3) According to Dancis et al (4) the intermittent form is probably also a distinct genetic trait In the leucocytes they have shown a decrease of decarboxylase activities in the fathers but not in the mothers of the affected children It is interesting to note that our loading experiment showed that intolerance to leucine was more pronounced in the patient s father than in his mother Also in both male siblings tolerance seemed to be less than in the patient s sister

Goedde et al (7) found that in intermittent BCKA either the female or the male parents can have decreased leucocyte decarboxylase activities They speculated on genetic allele models as a basis for the interpretation of the biochemical patterns in the patients and parents We must question however whether the leucocyte enzyme activities as they were determined until now, can really reflect genetic structures The highly variable findings obtained in the same patient by the latter authors suggest the contrary

The clinical aspects of classical BCKA have recently been discussed by Gaull (5) Attacks

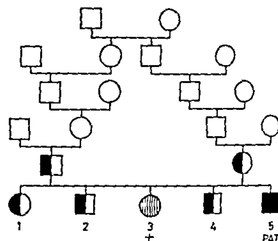


Fig 2 Pedigree of the patient s family ■ presumed homozygous male for variant of BCKA ◧ ◐ presumed heterozygous male and female □○ not tested

of this disease can be successfully treated with peritoneal dialysis as has been reported by Rey et al (12) Gaull concluded that a diffusible "toxic factor is responsible for the reversible neurological changes in this disease but this need not necessarily be the branched chain keto or amino acids The findings of Gerritsen & Milton (6) and Schulman et al (13) support this view their patients who permanently produced branched chain keto-acids did not show any neurological abnormalities Peritoneal dialysis may also be effective in severe attacks of intermittent BCKA which can be difficult to correct (In our patient the metabolic acidosis was corrected only after 60 hours)

The clinical picture of our patient roughly corresponded with that of the intermittent form of BCKA except that his mental development was clearly retarded at the time that diagnosis was made It was this alarming phenomenon connected rather with structural changes in the brain than with an acute intoxication that prompted us to continue the dietary treatment and not to stop the treatment as soon as the branched chain keto acidotic attack was over, as is usually advised In this way we hoped to avoid any further episodes of branched chain keto aciduria which might have aggravated the presumed brain-damage This danger existed for frequent respiratory infections oc-

curred during treatment. Moreover the patient accepted the diet very well and the treatment did not raise any difficulties at home We realize however that the favorable course of the boy s mental development is not necessarily a result of our prolonged treatment

SUMMARY

A case of branched-chain keto aciduria is described The boy s clinical picture was similar to that of patients with the intermittent form of this disorder but severe mental retardation was present

It proved possible to treat him successfully with a diet low in branched-chain amino acids and at the age of 3 years after 18 months treatment his mental development corresponded with his age

Details are given of the clinical course and the dietary treatment The findings in the patient s family are also described with the results of oral loading tests with l leucine performed on all members of the family

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LETTER TO THE EDITOR

To the editor

I am writing in response to the *report by* O Schweisguth et al, 'Bilateral Non Functioning Thecoma of the Ovary in Epileptic Children Under Anticonvulsant Therapy in *Acta Paediat Scand 60* 6–10, 1971

The authors have not demonstrated the ovarian lesions to be thecomas It is likely that the changes in the ovary are a result of partial or intermittent venous or lymphatic obstruction There are two reasons why the lesions of the ovaries are not thecomas First, bilateral thecomas as we found in a large study (*Cancer 21* 255 1968) are medical curiosities and reports of bilaterality are misleading and overstate the frequency of it Second, I reviewed the ovary slides in the second case, and found no evidence of thecoma There was swelling marked edema with focal hemorrhage but most important, retention of the outline of the ovary and preservation of follicular structures These findings indicate the lesions are ovarian enlargements not thecomas and are probably secondary to venous or lymphatic obstruction Such changes usually occur in children secondary to torsion The literature is beginning to reflect these observations (*Obstet Gynec, 34* 565 1969 and *Amer J Surg, 117* 726, 1969)

I am incorrectly quoted on page 9 of the article as rendering a diagnosis of thecal hyperplasia' In my correspondence with the authors on 3 April 1970 I stated

"The ovarian changes represent a rare alteration of the ovaries that occurs in association with torsion of the ovarian adnexal structures The ovaries become tremendously enlarged secondary to venous obstruction, there is marked stromal edema, stromal hemorrhage and thrombosis of capillaries and veins The lesion is nearly always found in a child, as is expected from the higher position of the ovaries in the pelvis with greater freedom of motion These ovarian changes have been regarded by some as torsion of a thecoma but the absence of any well defined outline of a neoplasm and retention of the ovarian outline (as seen in the smaller of the submitted sections) indicates that most are simply ovarian enlargements secondary to venous obstruction A copy of your gross description would be helpful in completing the pathological details of this interesting case"

Regardless of the exact nature of the ovarian changes in epileptic children taking anticonvulsant medication, the association is most interesting and the authors have performed a service by reporting it

Sincerely,

Henry J Norris, M D
Chief Gynecologic, Obstetric and Breast Pathology
Washington D C
USA

The Editor has asked Dr Schweisguth to comment on Dr Norris' letter to the editor

As long as the possible relationship between anticonvulsant therapy and the ovarian lesions is not determined, the true nature of these thecomas is debatable

But 1) The diagnosis of thecoma was confirmed in our first case by several trained pathologists as well as by Faber s pathologist

in the case reported by him The finding of fat in the cells was an argument favoring this diagnosis

2) To say that the ovarian lesions are associated with torsion and are due to veinous obstruction is—at least in our cases—a pure hypothesis which is not based on any surgical or gross pathological examination

To conclude we have added "non functioning to the term of "thecoma in order to emphasize the unusual presentation of these ovarian lesions We agree that further knowledge in the physio pathology is needed before a true nomenclature can be ascertained

Odile Schweisguth

PROCEEDINGS OF PAEDIATRIC SOCIETIES

FINNISH PAEDIATRIC SOCIETY

Meeting Febr 13, 1971

Martin Seip (Oslo, Norway) Fat and carbohydrate metabolism in generalized lipodystrophy

The most striking feature of generalized lipodystrophy is the extreme paucity of fat in adipose tissue, resulting in a great reduction in the size of the functioning adipose organ The lack of an adequate adipose organ leads to serious disturbances in fat and carbohydrate metabolism and, in most patients, to a markedly increased metabolic rate in the absence of hyperthyroidism These disturbances have been studied in 6 patients with the congenital form of generalized lipodystrophy

The fundamental defect in fat metabolism is the inability to store triglycerides in the adipocytes As a result a marked tendency to hypertriglyceridemia with increases of the pre-β and chylomicron fractions fatty infiltration of the liver and some deposition of fat in the reticuloendothelial system can be demonstrated Great fluctuations in plasma triglycerides are found depending on the dietary supply of fat and carbohydrate The peripheral utilization of fat is not impaired plasma FFA low normal or decreased plasma glycerol 3–6 fold increased

The alterations in carbohydrate metabolism seem to be secondary to the greatly impaired ability to store glucose as fat in the adipocytes During the first years of life glucose tolerance is normal, but from 8–10 years of age glucose tolerance rapidly decreases, and with cessation of growth at approximately 12 years of age frank diabetes without ketosis develops Some degree of insulin resistance is demonstrable already from infancy, and the insulin resistance increases with age The fasting plasma insulin levels are high especially in later childhood and the insulin responses to glucose and tolbutamide stimulation are exaggerated and prolonged After cessation of growth a partial exhaustion of the β cells seems to occur with somewhat lower fasting insulin levels and a relatively poor response to glucose stimulation Other aspects of carbohydrate metabolism (peripheral utilization of glucose hepatic lipogenesis from glucose etc) are not impaired

The hyperinsulinism combined with the availability of an excess of calories promotes anabolic processes resulting in increased rate of growth and skeletal maturation, muscular hypertrophy and splanchnomegaly

The hypermetabolism may perhaps be due to the ability of fatty acids to uncouple oxydative phosphorylation

These findings demonstrate most dramatically how important the normal adipose tissue is and the serious consequences of an inadequate adipose organ for a number of metabolic processes

O Koskimies

Acta Pædiat Scand 60 603–619 1971

PROCEEDINGS OF PAEDIATRIC SOCIETIES

EUROPEAN SOCIETY FOR PAEDIATRIC ENDOCRINOLOGY

Ninth Annual Meeting Lyon July 16–21 1970

H Greenberg P Czernichow R. C Reba J Tyson & R M Blizzard (Baltimore Maryland)
Observations on the maturation of thyroid function in early fetal life

Serum from twenty one normal human fetuses was obtained after therapeutic abortion for psychiatric indications Fetal crown–rump length ranged from 5 2 cm to 22 5 cm corresponding to the gestational age of 65 to 168 days

Serum thyroxine assayed by the Murphy Pattee method was identified in the second smallest fetus examined at 78 days gestation Thereafter it increased rapidly maintaining a significant linear correlation with crown–rump length until term ($r = 0.80$ $p < 0.001$) A similar rise was seen in TBG capacity ($r = 0.841$ $p < 0.001$) and in the affinity of TBG for tracer amounts of I–131 T4 ($r = 0.841$ $p < 0.001$) Free thyroxine also increased in a linear relation to gestational age until reaching term levels at 18 to 20 weeks ($r = 0.908$ $p < 0.001$) This increase occurred despite a rapid decrease in the dialysable fraction of thyroxine during this period

Radioimmunoassayable TSH was detected at 78 days gestation thereafter increasing rapidly to reach term levels by 16 weeks gestation After 100 days gestation levels were usually 4 0 μU cc higher than that seen in normal infants and children TSH did not correlate with total serum thyroxine but did correlate with free thyroxine in fetal sera

A significant fetal to maternal gradient of free thyroxine was demonstrated in fetuses less than 20 weeks gestation Fetal TSH levels were found to exceed that of the corresponding maternal TSH throughout most of the gestational period examined

P Olin S Almquist & R Ekholm (Stockholm)
Human fetal thyroid gland Influence of propyl thiouracil and of pituitary thyrotrophin

Previously we have shown *in vitro* synthesis of thyroxine and thyroglobulin in the thyroid glands from 10–11 week old human fetuses Prior to that time no iodide was incorporated into the thyroid although a thyroglobulin like uniodinated protein appeared at a somewhat earlier stage

The present studies showed that in the presence of a reducing substance as prophyl thiouracil 0 01 M radioiodide was concentrated 5–20 times into the thyroid tissue from fetuses above age 11 weeks without any iodination of thyroglobulin Protein synthesis as judged by ^{3}H leucine incorporation remained unaltered Thus the concentrating mechanism for iodide had developed at the time when the formation of thyroglobulin occurred

The *in vivo* effects of carbimazole on the human fetal thyroid gland was studied when a thyrotoxic woman on carbimazole treatment was subjected to a therapeutic abortion The thyroid glands from the twin fetuses crown–rump length 105 and 120 mm were normal judged by their light and electron microscopic morphology as compared with our observations on normal fetuses of corresponding age The

Table 1 *The conversion of androstenedione and dehydroepiandrosterone to testosterone by foetal testes in the presence of oxymetholone*

Foetal age (weeks)	Conversion to testosterone (dpm) From AD	From AD + Oxymetholone	From DHA	From DHA + Oxymetholone
6	6 828	6 253	10 618	7 653
8	*8 586*	*1 135*	*20 811*	*3 390*
8	14 552	6 261	50 170	7 432
9	5 285	2 384	11 096	6 826
12	6 568	3 133	18 115	3 680
22	50 143	3 770	42 358	16 389

in vitro incubation showed a normal synthesis of iodinated thyroglobulin Thus at fetal age 14–15 weeks the drug did not influence the structure of the thyroid nor inhibited the syn thesis of thyroglobulin *in vitro*

In order to clarify the relationship between the pituitary gland and the synthesis of thyro globulin the pituitary content of thyrotrophin was determined in a double antibody radio immunoassay Thyrotrophin was demonstrated in four fetuses 8–9 weeks old and in increasing amounts in fetuses more than 11 weeks old Thus pituitary thyrotrophin appears at the same time or earlier than the biosynthesis of thyroglobulin

W Hamilton (Glasgow) *The action of oxymetholone on human foetal testes*

Foetal testes were incubated with androstene dione (AD) and dehydroepiandrosterone (DHA) according to Hamilton et al (1) To the flask containing the right testis of each pair oxyme tholone was added in the same concentration as the substrates, while the left testis of each pair served as a control In Table 1 the in hibitory effect of oxymetholone on the syn thesis of testosterone from both AD and DHA is shown Thus, in keeping with other C2 sub stituted C19 compounds (2), oxymetholone blocks 3β hydroxysteroid dehydrogenase and 17β reductase The favourable anabolic/andro gen ratio of oxymetholone as an anabolic hor mone might be due to a similar effect in vivo

Reference

1 Hamilton W Grant J C & Wilson R R En zyme activity of human undescended testes *in* W Hamilton & F P Hudson (eds) *Errors of phenyl alanine thyroxine and testosterone metabolism* Livingstone Edinburgh 1970
2 Neville A M & Engel L L *J Clin Endocrinol Metab 28* 49 1968

N Josso (Paris) *Histo enzymatic study of the human fetal testis*

A histo enzymatic study of testicular tissue was made in fifteen fetuses whose gestational age ranged from 9 to 22 weeks Tissue fixed in al coholic Bouin fluid and in Gendre fixative, and fresh frozen tissue were studied

Before 12 weeks of gestational age the semi niferous tubules of the young fetal testis con tain primitive germ cells recognizable by their high alkaline phosphatase and glycogen con tent Acid phosphatase was the only other en zyme found in seminiferous tubules at that stage In the interstitial tissue NADH and NADPH tetrazolium reductase and glucose-6 phosphate dehydrogenase were found in the peripheral part of the testis Δ5–3β hydroxy steroid dehydrogenase was found in trace amounts 17 β hydroxysteroid dehydrogenase was not identified

After 12 weeks of gestational age primitive germ cells gradually disappear from the semini ferous tubules Alkaline phosphatase activity wanes and is no longer found at 17 weeks Glycogen disappears a little later at 19 weeks Enzymatic activities characteristic of post natal seminiferous tubules such as adenosine tri phosphatase 5 nucleotidase phosphorylase, are found in low amounts the activity of acid phosphatase increases Interstitial tissue is abundant and harbours intense activity of en zymes engaged in energy providing metabo lisms Steroidogenetic enzymes are also con centrated in the interstitial tissue Δ5–3β hy droxysteroid dehydrogenase 17 β hydroxy steroid dehydrogenase and secondary alcohol dehydrogenase

T Taylor & W Hamilton (Glasgow) *Activity of Δ⁵ 3β hydroxysteroid dehydrogenase isomerase in the human foetal adrenal*

It is currently postulated that the activity of this critical enzyme system is probably deficient for substrates pregnenolone and DHA during the first half of gestation. This concept is here reinvestigated in an *in vitro* rate study with post incubation quantitation by a double isotope dilution technique involving group isolation of product Δ4-3 oxosteroids acording to Taylor (1).

With each foetus a series of timed incubations (max 30 min) were performed with adrenal slices in Krebs Ringer bicarbonate buffer containing glucose using as substrate either ¹⁴C-4 pregnenolone (20 foetuses) or ¹⁴C-4 DHA (18 foetuses).

Most results demonstrated conversion rates of substrate to Δ4-3 oxosteroids in the range 0-0.2% /mg protein/min. Tissue from a virilizing adrenal carcinoma (child 9 years) which is generally considered deficient in this enzyme system gave values of 0.80 and 0.23% conversion mg protein/min from pregnenolone and DHA respectively, confirming that the foetal enzymic activity is probably low per unit mass.

However with pregnenolone adrenal glands from 3 foetuses out of 8 around 12 weeks of gestation gave high conversion rates ranging between 0.60 and 1.20 %/mg protein/min.

With DHA adrenals from 6 male foetuses and 2 of undetermined sex formed Δ4-3-oxosteroids, adrenals from all 6 female foetuses did not.

These results are too few for high significance but the tentative suggestion is made that a sex difference may exist in the conversion of DHA to Δ4-3-oxosteroids. If this is so it might be postulated that an adrenal role in foetal sex development or its maintenance could be operative.

W Teller & D Beckmann (Ulm) *Studies on the influence of thyroid function on steroid metabolism in newborns*

It has been shown that newborns excrete mainly tetrahydrocortisone (THE) rather than tetrahydrocortisol (THF) (1). At this age the ratio THF/THE is less than 0.1 compared with 0.4-0.6 in older children. Hellman et al. (2) as well as Gold & Crigler (3) were able to produce with thyroid hormones a shift of urinary steroid patterns towards 11 keto compounds in older individuals. In the paper to be presented the hypothesis was tested whether elevated levels of thyroid hormones (PBI) in newborns have similar influence on the preferred 11 keto pathway of steroid metabolism. Twelve apparently normal and healthy infants of both sexes were given 25 mg of propylthiouracil twice daily from the 3rd through the 9th day of life when a 24 h urine was collected for steroid analysis and blood drawn for PBI. Twelve untreated infants served as controls with urine collections and PBI determinations between the 5th and 7th day of life. The average levels of PBI were 8.7 μg per 100 ml in the untreated and 4.2 in the treated group. Steroid analyses were performed according to hydrolytic extraction and chromatographic procedures as reported previously (4). In the two experimental groups neither 11 deoxy C_{19} steroids (dehydroepiandrosterone, androsterone, etiocholanolone) nor 11 oxy C_{19} steroids (11 OH and 11-O etiocholanolone, 11-OH and 11-O androsterone) nor C_{21} steroids (tetrahydrocortisol, allo-tetrahydrocortisol, tetrahydrocortisone) showed appreciable differences. It is therefore concluded that in newborn infants the preferred 11 keto pathway of steroid metabolism is autonomous rather than influenced by thyroid hormone levels as is true in older subjects.

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3 Gold N I & Crigler J F *J Clin Endocr 23* 156 1963
4 Teller W *Z ges exp Med 142* 222 1967

R Wolter & C Delcroix (Brussels) *Serum growth hormone in normal adolescents*

Intravenous insulin tolerance tests were performed after a 12 hour fast in 60 normal prepubescent children and in 35 normal adolescents with all degrees of puberty Human growth hormone was measured in serum by double antibody immuno assay Wilhelmi HGH was used as standard

The mean curves of serum HGH response to insulin show higher values for adolescents than for children at the fasting level and during the whole test The highest level of HGH in serum in response to insulin does not vary with age before puberty The mean value is 17 4±4 9 (S D) ng/ml Half of the adolescents have higher maximal response than children with a range of 7 5 to 60 0 ng/ml There is no difference according to sex, height or stage of puberty

The disposal rate of unlabelled HGH (Raben badge 22) was studied in 8 children 4 adolescents and 4 adults HGH was given intra venously as a single injection A computer analysis of the disappearance curves was used to describe the experimental data by the sum of two exponential functions

The rate of disappearance of HGH is independent of the dose given The mean transit time of HGH ranges from 25 1 to 38 4 min in the children It is comparable in the adolescents with a range of 29 3 to 39 9 min Among the adults, one has a mean transit time of 29 0 min, but in the three others the range is 40 1 to 57 6 min

According to the date presented the higher levels of serum HGH observed in adolescents, as compared with children, cannot be related to a difference in the disposal rate of HGH It is thought therefore that the growth spurt of puberty is accompanied by an enhanced growth hormone release Adults may have a slower disposal rate of HGH

J C Job , P C Sizonenko & M Balage (Paris) *Étude statistique des resultats des epreuves de stimulation de la secretion hypophysaire d hormone de croissance par l insuline et l arginine*

Z Laron, I H Hochman, M Karp A Pertzelan & L Dolberg (Petah Tikva) *Effect of gonadotrophin therapy on testicular volume and sexual development in adolescent boys with hypogonadotrophic hypogonadism*

The effect of treatment with human gonadotrophins was evaluated in 6 hypogonadotrophic adolescent boys Treatment was initiated at 15 to $19\frac{10}{12}$ years of age for periods ranging from 3 to 25 months The dosage ranged from 1 000 to 10 000 I U /week for HCG and from 150 to 300 I U /week for HMG An increase in testicular volume during the course of HCG alone and especially during combined HCG–HMG therapy was registered There was also an effect on the secondary sexual characteristics but little influence on bone maturation

Due to the slow induction of secondary sexual characteristics and the cost and inconvenience of this treatment schedule it seems inadvisable to use this therapeutic approach routinely

Z Laron, M Karp A Pertzelan & M Burke (Petah Tikva) *The correlation between insulin growth hormone and glucose in the syndrome of pituitary dwarfism with high serum IR HGH*

Twenty eight patients with the syndrome of pituitary dwarfism and high serum immunoreactive (IR) HGH ranging in age from 6 months to 20 years were subjected to part or all of the following tests oral glucose tolerance arginine infusion, insulin tolerance Determinations of blood sugar plasma insulin and growth hormone were performed concomitantly In addition several patients having the hereditary type of this disease underwent the same studies For control purposes served 17 children with pituitary dwarfism due to HGH lack (hereditary and sporadic) and 20 juvenile diabetics

It was found that both children with high biologically inactive HGH and those without HGH had low plasma insulin concentrations, which did not rise significantly during arginine infusion similar to many children with juvenile diabetes. During glucose load the response was variable Despite a lower insulin reserve they had no glucose intolerance most probably due to a metabolic "Houssay phenomenon" Interestingly, two of the parents heterozygots for the disease showed a prediabetic glucose intolerance

R. Illig, A. Prader A Ferrandez M Zachmann (Zurich) *Hereditary prenatal growth hormone deficiency with increased tendency to growth hormone antibody formation ("A type" of isolated growth hormone deficiency)*

Among 19 patients with isolated growth hormone (GH) deficiency who were studied by us during the last years 6 children could be distinguished by the following characteristic features

1 Small body length in relation to weight at birth less than 49 cm despite birth weights of 4 000 g in two and more than 3 500 g in two other children. This intrauterine growth retardation became more apparent when compared with their healthy siblings.

2 Retarded growth from birth which led to extreme dwarfism the growth deficit expressed in standard deviations was 7 3 (±1 0) at 2 years and 8 0 (±0 8) at 10 years The difference of growth deficit between these 6 children and the other patients with isolated GH deficiency is highly significant

3 Typical face with large vaulted forehead and small nose with retracted bridge

4 Strong anabolic action of GH during a probatory HGH therapy for 5–6 days (N retention test) 43 7% (±12.6) N retention compared with 31 3% (±1.5) in 6 other patients with isolated GH-deficiency ($p < 0\ 05$) and 15% in 21 control children.

5 Early appearance of GH antibodies followed by an almost complete arrest of growth after an initial short growth spurt. The antibody concentration and binding capacity were extremely high already a few months after beginning GH therapy antibodies could still be detected years after withdrawal of GH

6 Four of these patients were related to each other

These observations suggest that the 6 children suffer from a hereditary total GH-deficiency which is operative already before birth and which causes a lack of immune tolerance to homologous human GH

A Ferrandez A Prader & M Zachmann (Zurich) *Isolated growth hormone deficiency in prepubertal children height weight skinfold thickness skeletal age and cortical thickness of bone before and under treatment with human growth hormone*

Seven patients with isolated growth hormone deficiency aged 3 to 11 years were treated with human growth hormone (HGH) for a period of 0 8 to 1 2 years The effect on height weight skinfold thickness skeletal age as well as on the cortical thickness and on the diameter of the metacarpal bones were studied

If the changes of these parameters are expressed in standard deviations from the normal mean the following results are obtained

1 Before treatment height is the most retarded parameter (dwarfism) Weight is less retarded and the skinfold thickness is above the normal mean values (obesity) The retardation of the skeletal age and of the measurements on the metacarpal bones is about equal and clearly less marked than the retardation of height (no oesteoporosis)

2 Under treatment there is a rapid increase in height and cortical thickness a rapid normalization of the skinfold thickness and a moderate increase in weight indicating an augmentation of bone and muscle mass There is also a moderate increase of the skeletal age and of the diameter of the metacarpal bones

3 Gold N I & Crigler J F *J Clin Endocr 23* 156 1963
4 Teller W *Z ges exp Med 142* 222 1967

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The ratio of the combined length of hand bones over height varied from 35–43% in 9 individuals with male hypogonadotrophic hypogonadism (HH) Between individuals this ratio appeared to be independent of bone age (range 10?–19 years Tanner & Whitehouse method) of height (range 137–189 cm) and of treatment with HCG or androgens In individual cases the ratio underwent a slight rise over a number of years (maximum noted shift from 35 0 to 37 1 between ca 9 5 and 17 1 years and BA 10 2 and 15 4 years) Nine randomly selected cases of chromatin negative gonadal dysgenesis with heights ranging from 134–178 cm showed ratios ranging from 38 6–44 5%

Bone age of HH patients in their teens and early twenties remained arrested at 15–15 5 years as long as treatment had been limited to HCG Two untreated cases studied in their early thirties had bone ages of 18 years with open epiphyses of ulna and radius

Methyltestosterone was the first androgen received by most of the patients This drug had surprisingly little effect on bone age although pubic and facial hair growth were stimulated and growth in height was markedly accelerated Thus in one case treatment for 1 2 years resulted in a height increase of 9 2 cm while bone age advanced from 15 3 to 15 4 years Long acting mixtures of androgens (®Sustanon and ®Triolandren) appear to be more effective in stimulating bone maturation than methyltestosterone

Marked growth in height and in the length of the hand bones occurred during androgen treatment at bone ages of 15–15 5 years with a rapid and steep fall in the growth rate when the bone age exceeded 16 years

B Weber H Helge & E Werner (Berlin) *Pituitary tumour with hypersomatotropism precocious puberty and generalized melanosis following bilateral adrenalectomy for Cushing's syndrome*

In children Cushing's syndrome is rarely due to ACTH-dependent adrenal hyperplasia Even less frequently therefore a postadrenalectomy hyperplasia of the pituitary which occurs in about 10% of adult subjects under these circumstances may be observed in children

K K male now 14 years old developed hypercorticism at the age of 6 years Elevation of the already high urinary values of 17 OHC and 17 ketosteroids by metopirone and the histological finding of a diffuse non nodular adrenal hyperplasia after total adrenalectomy indicated an increased endogenous ACTH secretion and the existence of an apparently normal regulatory mechanism Postoperatively catch up growth and a generalized cutaneous melanosis occurred Before the age of 9 years precocious pubertal changes of the testicles secondary sex characters and the skeleton were observed Radiographically in 1966 an enlargement of the sella turcica was first noticed which appeared to be unilateral an increase progressively Impairment of vision or the visual field so far did not occur

Studies of the pituitary function revealed

1 Excessively elevated ACTH plasma levels[1] of 6 000 to 10 000 pg/ml throughout the day (normal 15 to 75 pg/ml), which however were still suppressible by high doses (8 mg/day) of dexamethasone (The generalized pigmentation may be due to an overproduction of both ACTH and MSH)

2 Increased values of urinary gonadotrophins before the age of 9 years

3 High plasma values of GH (40 to 80 ng/ml) following different stimuli normal suppression by oral glucose application

4 Normal TSH plasma values and normal thyroid function

These results indicate a hypothalamic rather than a primary pituitary disorder to be responsible for the development of the pituitary tumour Precocious puberty and increased GH secretion may be regarded as hormonal overlap reactions

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[1] Courtesy of Dr Ratcliffe London (radio-immunoassay) Dr Hammerstein Berlin (bio assay) and Dr [illegible] Paris (radio-immunoassay)

P Czernichow, A H Greenberg & R M Blizzard (Baltimore) *The metabolic clearance rate (MCR) of thyrotropin*

A constant infusion technique for studying the MCR of thyrotropin was developed In a preliminary study ¹³¹I-HTSH and an immunologically pure unlabelled HTSH were injected simultaneously into 2 surgically hypophysectomized patients and serum specimens were collected over a 6 hour period The specific activity of labelled to unlabelled hormone and the respective halflives established that the hormones were metabolized identically The half-life was 40 and 41 min for both patients The volume of distribution was 1 9 l and 3 2 l which was 8 8 and 7 1 % of total body weight The calculated MCR was 56 2 and 53 9 l/24 hr/m

The MCR was then studied using a constant infusion method The mean MCR of 6 normal subjects was 34 4 l/24 hr/m² (range 24 4 to 46 1 l/24 hr/m²) Two clinically euthyroid patients with Hashimoto s disease and an elevated TSH, and one partially treated hypothyroid were in the normal range (42 4, 46 6 and 32 6 l/24 hr/m² respectively) Two patients with clinical myxedema measured 9 8 and 15 1 l/24 hr/m², well below the normal range One patient with severe alcoholic cirrhosis and hepatic failure had a normal MCR (44 5 l/24 hr/m²) and one patient with chronic renal failure was below the normal range (19 1 l/24 hr/m²) The secretion rates were not available at the time this abstract was submitted

The effects of dexamethasone (D) on the MCR of TSH was studied in 7 patients Eight mg of D was administered over a period of 48 hours In 5 subjects the MCR was measured by constant infusion before and at the end of the treatment period and in 2 after withdrawal of the drug for 30 hours No changes were observed in any subject except for one hypothyroid who showed a small increase while on D In addition no consistent change was observed in the thyroxine TBG or TBPA levels although a slight decrease in the free thyroxine was noted after 48 hours on D With the previous observation of Wilbur & Utiger (*J Clin Invest 48* 2096, 1969) that D suppresses serum TSH levels and that TRF-induced TSH release is not influenced by D it was concluded that the effect of D on TSH secretion was primarily at a suprahypophyseal level

J M Tanner, H Goldstein & R H Whitehouse (London) *Standards for height of boys and girls making allowance for parental height*

Children referred to a paediatrician for confirmation of a diagnosis of abnormally small stature not infrequently have small parents Suppose the child is a little below the third centile of height in the regular standards, but that the parents average about the tenth centile for adults Clearly this child is less likely to be abnormal than if the parents averaged the fiftieth centile Some allowance should be made but it is not clear at present how much

A chart will be presented which enables the paediatrician to read off the centile position for a child in the age range 3–9 years when proper allowance is made for parental height Examples of its use will be given The data from which it is constructed are the regressions of child s on parents height for some 95 girls and 135 boys whose parents had actually been measured

These new standards are considerably more powerful than the regular ones that is they pick up abnormals better Because of the greater accuracy these standards afford it turns out that a child at the fifth centile on the regular parent unknown standards falls well below the third centile when it is known that his parents are at the fiftieth centile

J J van der Werff ten Bosch & J J M Platenkamp (Rotterdam) *Skeletal growth and maturation in male hypogonadotrophic hypogonadism*

A study is being made of the growth in length of the 19 long bones of the hand, maturation of the hand and wrist bones and statural growth and the effects of treatment

ko skeletal age 4 years) presenting the clinical features of hyposomatotropism was studied

Basal plasma growth hormone levels were between 60 and 76 μU/ml and rose to a maximum of 508 μU/ml during arginine infusion and to 395 μU/ml during insulin induced hypoglycemia Thyroid and adrenal function were normal Plasma growth factor activity was low Short term treatment with human growth hormone in dosage of 0 5 2 0 and 8 0 mg/m /day resulted in no or minimal effect on blood glucose plasma F F A urinary Ca/P ratio N creatinine ratio and OH proline excretion Plasma growth factor activity was minimally if at all affected by the treatment

Contrary to the patients described by Z Laron (1) who are supposed to produce an abnormal growth hormone molecule this case is thought to be the first demonstration of a deficiency located at the level of the induction of plasma growth factors

Reference

1 Laron Z *Israel J Med Sci 4* 883 1968

M Menking J Wiebel & W Blunck (Hamburg) *Treatment of precocious puberty with chlormadinonacetate*

The effect on linear growth bone maturation and sexual development of long term treatment with Chlormadinonacetate (CMA) in doses of 6 to 12 mg per day was observed in 9 girls (group A) with idiopathic precocious puberty and 4 patients (group B 2 boys) with precocious puberty associated with other disorders Mean duration of therapy was 32 8 months (range 15 to 60 months) for the two groups combined 28 7 months for group A and 42 months for group B

Pretreatment vaginal smears showed marked estrogenic stimulation Prolonged therapy resulted in atrophy of the mucosa Uterine bleeding ceased Mean acceleration of bone age over height age of all 15 patients was 15 5 months before and 13 8 months after therapy Respective figures were 15 2 months before and 16 3 months after treatment in group A 15 0 months before and 8 0 months after treatment in group B No significant undesirable effects were observed The difficulties of evaluating the effect of CMA on prognosis of adult height are discussed

M David A Ruitton & G Tell (Lyon) *Insulin secretion in eight cases of precocious puberty treated with medroxyprogesterone acetate*

Eight girls treated with Medroxyprogesterone acetate for precocious puberty (7 cases idiopathic 1 case pineal tumour) received an oral dose of glucose (1 40 g/kg body weight) The glucosemia insulinemia and plasma levels of NEFA were determined throughout the trial

In 3 cases the glucosemia rise was above that of normal In all cases insulin secretion was higher than normal In 5 cases the peak was elevated (148 130 106 110 86 μU/ml) and early b fore 45 min In 2 cases an important hyperinsulinemia was observed with a maximum peak of 440 and 263 μU/ml. In 1 case the insulin response comprised two peaks 86 and 67 μU/ml

In all cases the surface of the plasma insulin curve was remarkably large The fall of NEFA at 90 min was correct lower by 34% of the initial value in 4 cases but insufficient in 3 cases In 1 case the NEFA did not rise again at the end of the trial

The importance of the observed abnormalities was directly related to the duration of treatment the dose administered and the weight gain which was observed in all patients

In one case after 6 months of treatment the insulin response was already abnormal

The risk and severity of such a treatment is discussed in the light of these findings

H J Degenhart H K A Visser & H Boon (Rotterdam) *A study of the cholesterol splitting enzyme system in normal adrenals and in adrenal lipoid hyperplasia*

A severe clinical picture is seen in patients with congenital adrenal lipoid hyperplasia. Almost

P H W Rayner & B T Rudd (Birmingham) *The use of clomiphene citrate to assess pituitary gonadal function in males with delayed puberty*

Clomiphene citrate, a non steroidal oestrogen analogue, is capable of inducing the release of luteinizing hormone (LH) from the pituitary in normal adult males A preliminary assessment of the value of this response as a test of the pituitary Leydig cell axis has been performed in boys with delayed puberty or in whom abnormal pubertal development was anticipated

The urinary and plasma testosterone to the oral administration of Clomiphene citrate (100 mg once daily for 6 days) has been measured by a competitive protein binding assay in 12 male subjects (age range 9–22 years) Measurements were continued for 2 days after the drug was stopped

The following diagnostic categories were studied

Hypogonadotrophic hypogonadism (2 patients), Isolated pituitary GH deficiency (2) Constitutional short stature with delayed puberty (3), Prader Willi syndrome (2), Cryptorchidism (2), Penoscrotal hypospadias (1)

Basal urinary testosterone levels were below the normal pubertal range (0 4–0 6 μg/24 hr) in all except 2 patients Both hypogonadotrophic patients and both patients with the Prader Willi Syndrome showed no response The 2 youngest patients aged 9 and 11 years, also showed no significant response The remaining 6 patients showed maximal urinary testosterone levels after Clomiphene stimulation ranging from 4 7 to 36 4 μg/24 hr indicating a normal Pituitary–Leydig cell axis in 3 patients the maximum response occurred 2 days after Clomiphene was discontinued There was an increase in the maximum urinary testosterone levels recorded after Clomiphene with increasing chronological age in increasing sexual maturity

These results demonstrate that Clomiphene citrate administration may provide a basis for a clinically useful test of Pituitary–Leydig cell axis in males with pubertal abnormality Further studies are required during normal puberty and of the dose and duration of Clomiphene required to obtain an optimum response

D Schonberg (Tubingen) *Hamartoma of median eminence in precocious puberty A new neuroradiological technique to in vivo diagnosis*

The onset of complete isosexual precocious puberty before the second year of life with neurological signs such as convulsive disorders and behavioral changes suggests brain tumour and especially hamartoma of the median eminence to be the cause Neuroradiology is the main diagnostic tool in these cases Although more than 40 patients with precocious puberty by hamartoma have been reported only a few detailed neuroradiological studies *in vivo* have been published (1–5) After poor results with normal fractionated pneumencephalography a new and simple technique of directed air filling under permanent visual control was developed Deep sedation with full cooperation of the children was achieved by Droperidol, a new neuroleptic agent In 3 cases of early onset precocious puberty with epileptic seizures mental retardation, hypermotility and unexplained laughing spells hamartomas of the median eminence were shown by pneumencephalography In 2 cases an elevated level of gonadotropin releasing factor activity supported our diagnosis

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J L Van den Brande M V L Du Caju H K A Visser, W Schopman & H J Degenhart (Rotterdam) *Primary plasma growth factor deficiency (sulfation factor and thymidine factor) presenting as hyposomatotropic dwarfism*

A patient with severe dwarfism (chronological age $10\frac{2}{1}$ years height 85 cm weight 14 0

tisol (F) and 11-deoxycortisol (S) were very low whereas deoxycorticosterone (DOC) and corticosterone (B) secretion rates were increased 7 fold Results expressed as mg/m per day were as follows F 1 3 S 0 023, DOC 0 35 B 16 (mean normal values were F 7 5 S 0 76 DOC 0 055 B 2 2) Plasma gonadotrophins were markedly increased (FSH 106 LH 364 mIU/ml) Testicular biopsies revealed interstitial cell hyperplasia and early spermatogenesis The karyotype was 46/XY The pedigree showed no other affected members At laparotomy ovaries uterus and fallopian tubes were absent vas deferens was incomplete prostate was present External genitalia consisted of small phallus bifid scrotum third degree hypospadias and small vagina At puberty there was no growth of body hair or phallic enlargement Biopsy of marked gynecomastia showed both ducts and acini Testosterone administration produced virilization Sexual ambiguity demonstrates strong dependence of external genitalia on androgens for male differentiation Suppression of mullerian structures occurred despite female levels of testosterone indicating that this step in male differentiation is not testosterone-dependent Pubertal breast development in this male supports the concept of femaleness during ontogeny unless counteracted by male hormone Diagnosis of other adrenocortical enzymatic deficiencies is excluded by the steroidal studies The clinical response to testosterone excludes testicular feminization Deficiency of 17 hydroxylation must be added to the cause of male pseudohermaphroditism

M Zachmann J A Vollmin G Schachenmann & A Prader (Zurich) *11β hydroxylase deficiency Longitudinal steroid study in an infant*

In a neonate girl (46 XX) with marked clitoral hypertrophy and 11β hydroxylase deficiency was diagnosed at the age of 1 week Without treatment the steroid excretion was followed for the first year of life

The total 17 ketosteroids were slightly elevated hydroxylase deficiency could be excluded (pregnanetriol normal pregnanetriolone absent) The total 17 hydroxycorticoids (17 OHC) were first slightly then markedly elevated The excretion of testosterone was high While androsterone aetiocholanolone and dehydroepiandrosterone were detectable no 11 keto or 11 hydroxy 17 ketosteroids were found The excretion of tetrahydro-S (THS) was elevated already with 1 week A gradual increase was noted up to the age of 11 months and within the following 2 months a dramatic increase took place Tetrahydrodesoxycorticosterone (THDOC) was not detected at all up to 11 months and at 12 and 13 months was only found in small amounts This indicates that the 11β hydroxylation of S to cortisol is impaired while that of DOC to corticosterone appears to be intact After administration of metyrapone the 17 OHC and THS did not further increase Under ACTH there was a considerable increase of both 17-OHC and THS Our results demonstrate that there are either two 11β hydroxylating systems in the human adrenal or that one enzyme has two different receptor sites for compounds which are and are not 17 hydroxylated In addition this case shows that even in a marked deficiency the characteristic pattern with respect to THS appeared only after 1 year In this patient 16α hydroxy pregnenolone (16-OH P) was detected in urine up to 6 months of age At 13 months when the excretion of THS became very high 16-OH P was no longer detectable It is conceivable that the physiological insufficiency of the 3β hydroxysteroid dehydrogenase and the physiological preferential 16α hydroxylation have prevented THS to reach high levels during the first year of life

C C Forsyth (Dundee) *Adrenocortical atrophy and diffuse cerebral sclerosis Biochemical studies*

A boy diagnosed as having Addison s disease due to idiopathic atrophy of the adrenal glands at the age of 7 years developed the first evidence of diffuse cerebral sclerosis at 8 years He died at 9 years 11 months

no synthesis of hydrocortisone, aldosterone and C_{19} compounds seems to occur in these cases, while large amounts of cholesterol and other sterols are found in the adrenals of the patients (1) A defect in the conversion of cholesterol to C_{21} and C_{19} compounds is the most likely explanation

The enzyme system that converts cholesterol to pregnenolone has at least three activities (i) 20α hydroxylase, (ii) 22R-hydroxylase, and (iii) lyase (20αR-dihydroxycholesterolisocapronaldehyde-lyase) (2)

Incubation studies with extracts prepared from acetone defatted adrenal cortex mitochondria gave the following results No measurable conversion of labelled cholesterol into pregnenolone (< 0 01 %) was obtained with the post mortem tissue from a child with adrenal lipoid hyperplasia (3) yet 20α hydroxycholesterol on the contrary, was converted into pregnenolone for about 3 % This is completely comparable with normal tissue stored at −20°C for the same period The conclusion is that in adrenal lipoid hyperplasia a defect in the cholesterol-20α-hydroxylase is very likely Without 20α hydroxylation splitting of the side chain between C_{20}–C_{22} or C_{21}–C_{20} is improbable, so that neither C_{21} steroids nor androgens can be formed This agrees with older clinical and chemical data

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J Perheentupa, O Janne & R Vihko (Helsinki) *3β hydroxysteroid dehydrogenase deficiency Steroid studies in an eight year old boy*

Studies were discussed of plasma and urinary neutral steroids, urinary estrogens, and bile acids of duodenal bile in an 8 year old male first seen at the age of 1 month because of ambiguous genitalia and salt loosing The findings of his neutral steroids have already appeared elsewhere (*J Clin Endocrinol 31* 162, 1970) In summary, these clearly established the specific diagnosis as 96 % of the total of 1 4 mg/100 ml of plasma steroids, and 95 % of the total of 103 9 mg/day of urinary steroids had 3β hydroxy Δ5 structure The most interesting finding was an unequivocal elevation of urinary androsterone and 5β pregnane 3α,17α, 20α-triol (1 4 and 1 5 mg/day respectively)

An analysis of the boys urinary estrogens (Professor Herman Adlercreutz) has further revealed a several fold increase in estriol (19 μg/day) All the three hormonal steroids from below the deficient enzyme step in the metabolic pathway were identified by gas liquid chromatography and gas chromatography mass spectrometry

The patient s bile was analysed by Professor Jan Sjovall of Karolinska Institutet He found completely normal bile acid composition qualitatively and quantitatively As the 3β hydroxy steroid dehydrogenase step is also necessary in the synthesis of bile acids the liver seems to have an adequate activity of this enzyme Presumably the increased amounts of estriol, androsterone and pregnanetriol are produced by the liver from the Δ5 steroids dehydroepiandrosterone and pregnenetriol, which circulate in markedly elevated concentrations

M I New (New York) *Male pseudohermaphroditism due to 17-hydroxylase deficiency*

This is the first report of a male with 17-hydroxylase deficiency resulting in male pseudohermaphroditism ambiguous external genitalia absence of male secondary sexual characteristics and gynecomastia at puberty Diagnosis was based on extensive studies of steroid metabolism including low urinary excretion of 17-ketosteroids and 17 hydroxycorticoids which did not increase after ACTH no response of very low plasma testosterone and dehydroepiandrosterone to ACTH or chorionic gonadotrophin low urinary aldosterone and plasma renin which increased after dexamethasone Secretion rates of 17 hydroxylated steroids cor

ment with supplementary sodium chloride (4 g per day) the patient did better but a definite improvement was first obtained with desoxy corticosterone acetate (25 mg intramuscularly every 3 weeks)

Further studies showed a low aldosterone secretion 7 weeks old 11 μg/24 hours and 10 months old below 1 μg/24 hours both on supplementary sodium and after sodium restriction. Cortisol secretion was normal (16 4 mg/m²/24 hours) and corticosterone secretion high (7 6 mg/m²/24 hours) According to this the urinary C_{21} corticosteroid pattern showed normal amounts of cortisol metabolites (THE ThF allo-THF) and increased amounts of corticosterone metabolites (THA THB allo-THB) Plasma levels of corticosteroids and renin and other urinary metabolites were also studied

Besides this adrenal lesion the patient has a chronic pyelonephritis with vesico-ureteral reflux on the right side

H. Gleispach (Innsbruck) *Results obtained by measurement of 17 KS with gas liquid chromatography (GLC) and the method of Zimmermann*

It is shown that the knowledge of the pattern of the different 17 KS is important for a correct judgment of their metabolism and a great help for a good treatment with drugs A specific method however must be used by the determination of the 17 KS Great differences are found between the values obtained by the photometric method of Zimmermann and the sum of the different metabolites received by GLC These differences are greater in children than in adults and are caused by chromogens which are formed by hot hydrochlorid acid hydrolysis and not received by enzymatic hydrolysis a following solvolysis at room temperature and continuous ether extraction The influence of these chromogens is demonstrated by the results obtained in the urines of two hirsute women where chromogens simulate high values of 17 KS Only high values for testosterone were found by GLC The response to an administration with drugs is more specific by GLC measurement. The importance of the knowledge of the 17 KS pattern is demonstrated by the following example A low excretion of 11 desoxy 17 KS increased the elevated excretion of 11 oxy 17 KS decreased in a child with AGS and salt losing syndrome when we used Florinef® as drug instead of cortisol derivatives

H Gleispach H Berger J Glatzl & P Heidemann (Innsbruck) *Puberty and the excretion of pregnanediol and pregnanetriol*

By our investigation of normal values of urinary steroids we found a high increase in the excretion of the pregnanes during puberty the increase of pregnanetriol being greater than that of pregnanediol This is in good correlation with our observation that the excretion of pregnanetriol increases more than that of pregnanediol under stimulation with HCG We made the same observation stimulating 2 patients with male pseudohermaphroditism a boy and a girl with precocious puberty and 3 boys with delayed puberty Patients with gonadal dysgenesis showed a low base excretion of pregnanes after stimulation with HCG the slow increase in the excretion was greater for pregnanediol than for pregnanetriol Gonadal testosterone could not be stimulated in 2 boys with Klinefelter s syndrome with an elevated excretion of pregnanetriol and a normal one for pregnanediol though it could be stimulated in 2 other cases of Klinefelter's syndrome with a normal base excretion of the pregnanes

D Knorr (Munchen) *Determination of plasma testosterone as hexadecafluorononanoate by GLC and electron capture detection*

Two independent methods are useful for the determination of plasma testosterone The competitive protein binding technique and gas liquid chromatography (GLC) using the electron capture detector (ECD)

The advantage of the GLC–ECD is the high specificity in low levels Heptafluorobutyrate is

There are twelve fully documented reports in the literature of boys with the combination of adrenocortical atrophy and diffuse cerebral sclerosis The clinical and pathological aspects of his case were outlined briefly as a background to a consideration of the detailed studies of adrenal function made during life and of the biochemistry of the brain at post mortem During life, six 24-hour samples of urine over the period of the boy s illness were assayed for dehydro epiandrosterone aetiocholanolone and androsterone, 11-hydroxyaetiocholanolone, 11-hydroxyandrosterone, 11-oxo aetiocholanolone 11-oxo androsterone There are no reports to date of such a study in this condition or in Addison s disease In addition, assays were made of tetrahydrocortisol, allotetrahydrocortisol, tetrahydrocortisone tetrahydrocorticosterone, allotetrahydrocorticosterone and tetrahydro-11-dehydrocorticosterone These steroids have been examined once in this syndrome by Dr M Damkjaer Nielsen of Copenhagen and they have been examined in Addison s disease by Dr Nielsen and by Dr W S Cost of The Netherlands The findings in this boy were compared with their work The brain biochemistry was investigated by Professor J N Cumings of the Department of Chemical Pathology, The National Hospital, London and shed further light on the nature of the syndrome which is a specific entity The two likely theories of causation involve either an error of metabolism common to the adrenal cortex and the brain, or the possibility that both the adrenal and brain pathology are due to an auto immune disorder Evidence for these theories was presented and reference was made to the likely mode of inheritance of the condition

J Girard, H R Hirt, U Buhler & M Vest (Basel) *Antibodies to the biologically active amino acid sequence of corticotrophin in an infant treated with synthetic β^{1-24} corticotrophin*

An infant treated with synthetic β^{1-24} corticotrophin (Synacthen CIBA) developed antibodies against the biologically active part of the ACTH molecule Subsequently the sera of 5 other children—for different reasons on long term ACTH treatment with Synachten —were investigated, and antibodies detected in 2 of these 5 patients With a radioimmunological method the binding of ACTH to the patients sera was identified as an antigen antibody reaction All sera were found to have a high avidity and a binding capacity of over 1 µg of corticotrophin or Synacthen respectively per ml of serum The possible danger of inducing antibodies against the biologically active part of the ACTH molecule by long term treatment requires a careful control of steroidogenesis and the sera should periodically be checked for antibodies

K H Kristensen, K E Petersen E Thamdrup & N C Hansen (Copenhagen) *Selective hypoaldosteronism Report of a case in an infant with determinations of secretion rate for aldosterone cortisol and corticosterone*

The patient a boy with a birthweight of 3 350 g was born by normal delivery after a normal pregnancy The parents were young and healthy, but their first child, a boy died suddenly 5 weeks old with the same clinical symptom as the patient

The patient was seen in the hospital when 3 weeks old with poor thriving but no vomiting at 20 days old the weight was 130 g below birthweight On admission the findings were Signs of weight loss dehydration, hyponatremia (serum Na 115 mEq/l) hyperkalemia (serum K 7 0 mEq/l) and acidosis (serum HCO_3 15 9 mEq/l) External genitalia had a little more pigmentation than usual in Scandinavian infants, the penis seemed to be enlarged (50 × 12 mm)

Urinary 17-ketosteroid excretion was normal (0 1–0 2 mg/24 hours) and it was not possible to detect pregnantriol in the urine Congenital adrenal hyperplasia therefore seems excluded but the salt loss could be due to hypoaldosteronism as first described by Visser On treat

was d v loped In this an aliquot of a 24 hour urine collection was subjected to hot hydrolysis at neutral pH for 6 hours (Fotherby 1959) Liberated steroids were extracted with diethyl ether and the ketones were separated from the non ketonic compounds by Girard reaction DHA was separated by paper chromatography in Bush A system and after eluting the concentration of DHA was measured spectrophotometrically by Zimmermann reaction (James & De Jong 1961) The overall average recovery of the procedure was 85 where DHA 7α-T(n) sulphate potassium salt added to the urine and taken all though the analysis The identity of DHA was confirmed after making various chemical derivatives and checking up their chromatographic mobilities against derivatives prepared from authentic steroids

Wh n this procedure was applied to the urine from 10 children aged 9–15 years with various adrenocortical disorders the levels of DHA were found to be above normal Of these 10 patients 2 had adenoma 4 had carcinoma and 4 had hyperplasia the value of DHA was between 2 8 and 5 1 mg/24 hr (mean 3 6 mg/24 hr) All these patients had to undergo surgery and in each case DHA was estimated after 3 months and the value of DHA was found to be between 0 38 and 1 1 mg/24 hr (mean 0 69 mg/24 hr)

From these results it is possible to conclude that urinary DHA determination could be used for the diagnosis of adrenocortical disorders in children

C E Gray & W Hamilton (Glasgow) *Excretion of dehydroepiandrosterone in cystic fibrosis*

The abnormality of the mesonephric structures is described in patients with cystic fibrosis (CF) by Kaplan et al (2) suggests that the intrauterine stimulus for their development might have been abnormal This has led to a consideration of dehydroepiandrosterone (DHA) and its ester dehydroepiandrosterone sulphate (DHAS) in patients since DHA as a precursor

Patient Age	Sex	Clinical rating	17 OX (mg/24 hr)	DHAS (μg/24 hr)	Normal DHAS	Normal DHAG
18	♂	Severe	2 5	30	392	65
20	♂	Good	4 7	187		
8	♂	Good	0 28	46 9	42.7	54 8
10 5	♂	Severe	0 12	5 6		
7	♂	Severe	0 44	13 6	6 29	4 96
7	♂	Good	0 6	13 5		

These results could indicate a reduced synthesis of DHAS by the adrenal cortex a reduced release of formed DHAS from the adrenal or defective resulphation of free DHA at the hepatic and/or intestinal levels

of testosterone is important in the genital development of the male foetus

DHAS and dehydroepiandrosterone glucuronoside (DHAG) were extracted from urine of patients according to the method of Fotherby (1) and Kellie & Wade (3) respectively Quantitation was by electron capture detection of DHA heptafluorobutyrate from a 9 foot hybrid column (7 ft 1° NGS 2 ft 1 ⁄ SE 30) against standard testosterone heptafluorobutyrate

Our studies of CF patients rated clinically as severe shows low DHAS levels compared to normal values reported by Loras et al (4) In all cases total 17 oxosteroids (17 OX) excreted daily are less than normal

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A J Hadjian M Cheddin & E M Chambaz (La Tronche) *Study of cortisol binding proteins by a competition technique and different graphical calculation methods Application to the neonatal period*

A competitive protein binding technique was used in order to study the characteristics of

the most common derivative of steroids for ECD–GLC

Kirschner (1969) described a new derivative, hexadecafluoronanoate, which is more than twice as sensitive in the ECD as heptafluorobutyrate The method includes the following steps Ether extraction of 10 ml plasma I Thin layer chromatography Derivation formation II Thin layer chromatography Gas chromatography with ECD
Correction for losses by a ^{3}H testosterone intern standard

Using this derivative we determined the plasma testosterone level in boys before and during the puberty Testosterone is excreted in urine also before the puberty deriving from adrenal androstendione Using the ECD–GLC technique testosterone was not detectable in plasma of prepubertal boys Normal values of plasma testosterone in childhood and in puberty will be presented

B T Rudd & B Morris (Birmingham) *Methodological difficulties in the measurement of C_{19} steroids by competitive protein binding (CPB)*

The asymptotic form of standard curves prepared from 3rd trimester pregnancy plasma is present over a wide range of dilutions and cannot be eliminated by dilution alone or isolation and use of the βglobulin fraction with a high affinity for 17βol steroids Adequate curves can be obtained when the total number of binding sites is low, as is demonstrable with plasma obtained 10 days post partum diluted 1 in 50 All plasma should be titrated for binding sites before deciding which is the most sensitive curve obtainable Highly diluted pregnancy plasma (1 300) does not keep at 4°C and yields curves with decreased binding affinity after 24–82 hours Prefrozen (−20°C) aliquots of undiluted plasma keep well without change in binding affinity Inadequate mixing of labelled plasma with solid phases to separate free from protein bound steroid, leads to flat curves Exposure time to solid phase is critical marked variation in time between paired analyses gives curves of poor precision

A major source of error is that due to an increase in method blanks from sources other than the biological material These are commercial binders in TLC plates, solvents used for elution e g ethanol, methanol ether which give values of apparent testosterone of 0 2–2 0 ng Every solvent used should be tested for quality in the assay, and plates prewashed Adequate TLC systems must be used to separate ' diols from testosterone, as they compete avidly for binding sites Enzymatic conversion of Δ^4 androstenedione to testosterone before assay requires rigid control of quality including $(NH_4)SO_4$ precipitation and acetone extraction as the enzyme itself contributes significantly to blank of the method

D Gupta, K Rager & R Huenges (Tubingen) *Studies on testosterone protein reactions in children in relation to age sex and different endocrinopathies*

The binding affinity (BA) and binding index (BI) of plasma proteins for testosterone were studied by a technique based on the principle of precipitation by dextran coated charcoal The binding parameters were examined in relation to concentration of both proteins and steroids Some of the factors that influence the binding capacity of the specific testosterone binding globulin were also investigated

Testosterone binding affinity levels were determined in children before and during puberty in relation to their sex and maturation Several other groups of children with various endocrine disorders such as precocious puberty, adrenogenital syndrome hypopituitarism hypogonadism and children receiving cyproterone were also studied

S B Pal & W Teller (Ulm) *Urinary excretion of dehydroepiandrosterone in children with adrenocortical disorders*

A systematic analytical procedure for the determination of urinary dehydroepiandrosterone

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A systematic analytical procedure for the determination of urinary dehydroepiandrosterone

fication of urinary steroids The high resolu ion on this type of column saves complicated prepurification steps The steroids are chroma ographed as trimethylsilyl ether or if an drenocortical side chain (20-one 17α ol 20-one 17α 21-diol) is present as methoxime tri methylsilyl ether derivatives For the identifica ion of unknown urinary steroids the same col umns are used in a combination of gas chroma ograph and mass spectrometer With a new echnique of splitless injection quantitative determination of urinary steroids are also pos ible The usefulness of this technique for quantitative analysis is illustrated by the ex ample of THS and THDOC In 16 children 0 1-14 years old) the excretion of THS under basal conditions ranged from <5 to 60 μg/24 hr and in 18 adults (16-36 years old) from 5 to 76 μg/24 hr whereas THDOC could not be detected After administration of metyrapone 6 normal adults excreted 990-8 700 μg/24 hr of THS and 120-1 100 μg/24 hr of THDOC

An untreated girl (1 1 years old) with congenital adrenal hyperplasia due to an 11β-hydroxylase deficiency excreted 2 800 μg/24 hr of THS and 32 μg/24 hr of THDOC Three untreated patients with congenital adrenal hyperplasia due to a 21 hydroxylase deficiency showed a slightly elevated THS excretion (90-154 μg/24 hr)

C G Bergstrand

cortisol binding proteins in human plasma After equilibrium, charcoal coated dextran was used to separate the free (F) from the bound (B) steroid From the values obtained for B F and total cortisol, different graphical representations were constructed for the identification of the binding species present in plasma and the calculation of the binding parameters for transcortin The Scatchard plot appears the simplest approach and yields satisfactory results for clinical research purposes as compared with more elaborate calculation methods (e g Baulieu E E & Raynaud J P *Europ J Biochem, 13* 293, 1970)

The values obtained for CBG in adult and pregnancy plasma are in agreement with the values found in the literature (6 to 8×10^{-7} M for the binding capacity and 4 to 7×10^{-8} l/M for the affinity constant Kt at 3°C) With this type of technique, the temperature appears to be the most critical point as it greatly influences the Kt value

The CBG characteristics obtained by this approach in infant and new born plasma are presented the affinity constant Kt was found similar to the adult values, whereas the binding capacity was always low ($\leqslant 3\ 5 \times 10^{-7}$ M) These low values were corrected to the adult levels within the first 3 or 4 weeks of life Low values were also found in the cord plasma as compared with the average adult binding capacity, this is in agreement with the previous finding by De Moor s group using gel filtration (De Moor et al *J Clin Invest 41* 816 1962)

Some technical difficulties encountered in the accurate measurement of cortisol basal level and subsequent binding studies in the new born and cord plasma are discussed In such plasmas containing high level of interfering compounds treatment with an adsorbent before the binding studies was found very useful

This type of approach results in a simple and relatively rapid method which may facilitate the study of various biological fluids Its results compared well with those obtained using equilibrium dialysis which may be considered as the reference method On the other hand, more information is obtained than with the simple gel filtration technique

At the same time measurement of plasma cortisol concentration by CPB using the patient plasma as the source of the binding protein might be carried out as described by Pegg & Keane (Pegg M J & Keane, P M *Steroids, 14* 705, 1969)

R Rappaport (Paris) *A technique of dosage of cortisol by competitive protein binding*

The technique of cortisol estimation by competitive protein binding analysis has been applied to measurements in plasma 0 2 ml plasma volumes were used for micromethod 0 07 ml for the ultra micromethod A careful control of the temperature during incubation, shaking and centrifugation allowed excellent reproducibility The temperature should not exceed 10°C at any moment, in order to obtain maximum binding of the steroid on the protein Separation was achieved by Fuller s earth (20 mg) The precision of the method was evalued by determining the coefficient of variation between 5 1 % and 8 1 % for the micromethod Known amounts of cortisol were added to a cortisol free plasma with a recovery close to 100% Normal values for men were $11\ 7 \pm 2\ 5$ μg/100 ml by the micromethod and $13\ 4 \pm 3\ 1$ μg/100 ml by the ultramicromethod ($r = 0\ 82$ $p < 0\ 0001$) For both methods we used human plasma at 2 5 % and 0 5 % dilution The standard error is 0 5 for a plasma concentration 6 3 μg/100 ml Use of the method is limited by its low specificity The ^{3}H cortisol displacement from the Fuller s earth was measured for 11 deoxycortisol corticosterone 11 desoxycorticosterone testosterone, prednisone and prednisolone Practical application for clinical investigation are discussed

J A Vollmin (Zurich) *Separation and identification of urinary steroids on glass capillary columns in a combination gas chromatograph mass spectrometer*

Glass capillary columns have been used for the gas chromatographic separation and iden-

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VIRUS SEROLOGY AND SERUM IgE LEVELS IN CHILDREN WITH ASTHMATOID BRONCHITIS

T FOUCARD T BERG S G O JOHANSSON and B WAHREN

From the Department of Paediatrics and the Blood Centre University Hospital Uppsala and from the Department of Virology National Bacteriological Laboratory Stockholm Sweden

The relationship between asthma and bronchitis with signs of obstruction (wheezing) has been discussed for many years Among children who react to respiratory tract infections with wheezing there is an overrepresentation of children who will become asthmatics Several retrospective studies (10–12 14 29 30 35 39 42) of such children and a few prospective studies (19 44) have shown an asthma frequency varying from about 5 to 50% These figures can be compared with the asthma frequency of about 1.5% among Swedish school children (26) and of about 5% among American college students (16)

In children with wheezing during respiratory tract infections it is often difficult in the single case to give a correct prognosis as to the risk of developing asthma Recurring symptoms higher age of onset allergic heredity and previous atopic manifestations are unfavourable prognostic factors (5 12 14 31)

In 60% of adults and older children with extrinsic asthma Berg & Johansson (3 20) have found a pathological increase in the serum level of immunoglobulin E A study of the IgE levels in infants with astmatoid bronchitis might therefore be of value in assessing the prognosis

During the last decade studies have shown that infections giving rise to asthma like symptoms in children are predominantly of virus etiology (1 6 28 37) Bacteria play a far more inferior role at least in the early stage (1) Of approximately 150 known airway viral pathogens there are only a few causing most of the infections accompanied by symptoms of bronchial obstruction The most common of these viruses are the respiratory syncytial (RS) virus and the parainfluenza viruses (6 28 37)

Whether the obstructive symptoms caused by RS virus infections are of a non specific mechanical nature (37) or have an immunological background (8 15) is still not established

The aim of this study has been to investigate the serum IgE levels in a group of children with asthmatoid bronchitis and relate these levels to the virus serology and other factors of prognostic interest

MATERIAL AND METHODS

Definitions

Asthmatoid bronchitis is defined as a condition with dyspnoea and expiratory wheezing in children with signs of respiratory infection *Bronchiolitis* in this work and as commonly done in Scandinavia is defined as a more severe clinical picture occurring especially in infants less than 6 months old accompanied by pronounced dyspnoea, tachypnoea, sometimes cyanosis hyperinflation of the lungs and marked suprasternal and subcostal retraction. Most of these infants have expiratory wheezing too Cases bordering bronchiolitis and bronchitis are not uncommon and may be difficult to evaluate In the Anglo Saxon literature the term bronchiolitis is sometimes used in a broader meaning including our term asthmatoid bronchitis Some authors have used an

BOOK REVIEWS

J Huter & B Zehnthbauer *Übergang von Medikamenten in die Muttermilch und Nebenwirkungen beim gestillten Kind* Georg Thieme Verlag Stuttgart 1970 80 pp DM 22.—

In literature there are very few monographs on the transfer of drugs from mother to infant via breast milk and on their secondary effects Therefore this book giving a systematical and comprehensive survey on this subject is welcome About two hundred of the more common and important drugs are discussed and for each drug the results of studies on animals or on man are briefly described The chapters dealing with the most common drugs end with a short clinical conclusion For further studies the reader is referred to 346 references

The book is of value in pediatric (neonatal) as well as in obstetric practice

Tor Lindberg

H G Schwarzacher & U Wolf (eds) *Methoden in der medizinischen Cytogenetik* Springer Verlag Berlin Heidelberg and New York 1970 186 pp illus DM 48.—

Clinical cytogenetics is a rapidly expanding branch of medical science Over the past 10 years it has developed into an important aid in the diagnosis of birth defects of various kinds it is also of interest for the oncologist Early research in this field employed complicated methods which called for the co operation of a tissue culture department The introduction of the blood culture method in 1960 made routine analysis of human chromosomes available also to less specialized laboratories and this is still the main method for clinical cytogenetics The presence of mosaicism for instance sometimes necessitates the analysis of other tissues too Autoradiographic analysis of DNA synthesis patterns in individual chromosomes often gives valuable information on the identification of abnormalities During recent years important studies of meiosis in man have been made and such studies are of increasing importance for the clinical analysis of the germ line of adult men and often help to clarify for instance translocations All these aspects are admirably covered by different authors in this book mainly from a methodological point of view The book is very comprehensive and well illustrated It discusses various technical modifications and gives detailed schedules for the use of these sometimes involved methods It should prove a very valuable manual for all engaged on cytogenetic research although no doubt many laboratories use further modifications and special techniques The rapidity of expansion in this field is illustrated by the lack of references to methods for prenatal chromosomal analysis using amniotic fluid cells and to methods for the identification of Y chromosomes in interphase nuclei

Bengt Kallen

O Oetliker (ed) *Nephrologie im Kindesalter I* Pädiatrische Fortbildungskurze fur die Praxis Vol 27 Karger Basel and New York 1970 117 pp US $7 20

R Frey & Th Baumann (eds) *Nephrologie im Kindesalter II* Pädiatrische Fortbildungskurze für die Praxis Vol 28 Karger Basel and New York 1970 114 pp US $7 20

The current volumes of this series are dealing with kidney diseases in childhood mainly the glomerulopathias and urinary tract infection

The books report papers from the annual congress of Schweizerischen Gesellschaft fur Pädiatrie giving both theoretical and practical aspects of the mentioned groups of diseases —The value of needle biopsy of the kidney is briefly outlined by J Ehrensperger Lausanne and the pathological changes in the glomeruli and their clinical significance more extensively discussed by R Habib Paris Experimental immunological lesions to the glomeruli as well as renal engagement in collagen diseases are questions elucidated by other authors —Pyelonephritis and malformations of the urinary tract diagnostic problems in urinary tract infection and post operative treatment of pyelonephritis in urinary tract infection are subjects treated In other papers is dealt with basic renal physiology radiologic findings in childhood urinary tract disease and tubular dysfunction

P Royer Paris contributes with an analysis of the renal hypertension in children and a valuable survey of renal disease in the newborn and in early infancy

In those two small books in all 16 papers are presented 6 in French and 10 in German There are no multilingual summaries Most papers are informative although short and the books give a rapid and actual orientation of the subjects mentioned

Sture Sjoblad

with the CF technique In addition to these children another two children 9 and 15 months old showed the same titer rise against RS virus but these cases have been recorded only as ECHO virus infections since at least a 10 fold titer rise was found against ECHO 3 and 7 respectively

For the cases recorded below the zero line as possible parainfluenza virus infections the titer changes were of another type In the first serum sample they had titers ranging from $^1/_{20}$ to $^1/_{160}$ but in the second sample the titers had decreased in almost all cases In the third serum sample the titers had decreased significantly compared to the first sample in all analyzed cases Treatment of these children s acute sera with dimercaptoethanol gave no reduction of the titers indicating that the antibodies were not of IgM nature

Almost all RS virus infections occurred in infants younger than 15 months of age In this age group significant titer rises against RS virus were demonstrated in slightly more than 50% of the infections where a virus etiology could be shown For infants who were less than 9 months of age the corresponding figure was 75% Significant titer rises to adenovirus were found in sera from only 3 children who were 21–33 months old Two of these children also showed a significant titer rise against another virus

RS virus and parainfluenza virus infections showed an uneven distribution during the investigation period (Fig 2) Parainfluenza virus infections (type 3) were common during the first winter The first RS virus infections were noted in May–June 1969 and during the following autumn and winter this virus was found to be the dominant infective agent



Fig 2 Distribution of virus infections during the investigation period (serological diagnosis) in 72 infants with asthmatoid bronchitis. Symbols as in Fig 1

Table 1 *Virus serology findings in 72 infants with asthmatoid bronchitis 'Possible infections' means infants with titer rises less than 4 fold for RS virus or high initial titers for any virus*

Virus	No of cases with ≥4 fold titer rise		Additional cases with possible infections		Negative serology
RS	13		2		
Parainfluenza	5		12		
Type 2		1		2	
Type 3		4		10	
ECHO	13				
Type 3		3			
Type 6		2			
Type 7		1			
Type 9		4			
Type 11		3			
Adeno	3		1		
Influenza A	1				
	35 (48)		15 (21)		22 (31)

In two cases a simultaneous titer rise was found against two different viruses in one case RS virus + adenovirus and in the other ECHO virus + adenovirus

Table 1 summarizes the serologically demonstrated virus infections RS virus infections were most often demonstrated (18%) The total number of ECHO virus infections was as high but these infections were distributed among 5 different types

Swabs from nose and throat for routine bacterial culture (47 children) showed a growth of potential airway pathogens in 14 cases Of these 1 had *Hemophilus influenzae* 4 had *Hemophilus parainfluenzae* 2 had *β hemolytic streptococcus* 1 *Klebsiella* and 6 *Staphylococcus aureus* Three of these 14 children showed significant titer rises against RS virus or adenovirus

The clinical pictures white blood cell counts and sedimentation rates were rather similar for the different kinds of virus infections The RS virus infected infants had fever (≥38 C) more often than other virus infected infants

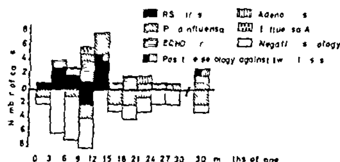


Fig 1 Virus serology in 72 infants with asthmatoid bronchitis Symbols above the zero line indicate at least 4 fold titer rises Filled symbols below the zero line indicate titer rises less than 4 fold for RS virus or high initial titers for any virus

upper age limit of two years (10 12 44) others have not (19 37 42) *Atopic manifestations* include hay fever extrinsic asthma certain cases of eczema and occasional cases of urticaria

The series consists of all but two children with asthmatoid bronchitis hospitalized for a few days at the Department of Paediatrics University Hospital Uppsala during the period November 1968 to March 1970 (49 children) Besides 23 children treated as outpatients during the same time are included In total there were 46 boys and 26 girls Forty four of the children (aged 2–33 months) had their symptoms for the first time while 28 children (aged 5–66 months) had had one or more similar attacks earlier These former attacks had all occurred in connection with respiratory infections according to the parents All the patients were seen by one of the authors (T F)

In the hospitalized children the blood samples were obtained by venipuncture during the first 24 hours In a few cases the sampling was delayed until 48 hours A second sample was taken from all patients 2–4 weeks later (mean 20 days) and a third sample another 3–4 months later After centrifugation of the clotted specimens the sera were withdrawn and kept frozen at −20 C until analyzed

White blood counts total eosinophil counts and sedimentation rates were done in the acute phase in all but a few of the outpatients Some additional investigations were performed on the hospitalized children nose and throat swabs for routine bacterial culture smears of bronchial or nasal secretions for examination of eosinophils and pulmonary X rays

Immunoglobulins

Quantitative determinations of the immunoglobulins G, A M and D were done on the sera of the first 30 patients by single radial immunodiffusion in agar gel according to Mancini et al with minor modifications (23) The IgE concentrations were measured by the radio immunosorbent technique (RIST) of Wide & Porath (40) as applied for IgE determination by Johansson et al (21) The mean error of the method calculated as the standard deviation (S D) in duplicate determinations was 14% The IgE concentrations obtained were expressed as percent of the arithmetic mean value for healthy children of the same age according to Berg & Johansson (2 22)

Reduction with 2 mercaptoethanol

0 4 ml of serum diluted 1 4 was mixed with 0 05 ml of 0 2 M 2 mercaptoethanol and incubated for 1 hr at 37° 0 05 ml of 0 4 M iodoacetamide was added to prevent reaggregation (13) The supernatant was used for assay

Complement fixing (CF) antibodies

The following antigens were used for complement fixation influenza viruses A 2 and B RS virus adenovirus type 6, parainfluenzaviruses types 1 2 and 3 all prepared by Dr M Grandien National Bacteriological Laboratory Stockholm Serum titrations were made in doubling dilutions starting with 1 5 by the microtiter method (36) A 4 fold titer increase or more was considered significant Patients with titers ≥40 in the acute phase or significant titer decreases between the first and the second sample were classified as possible infection as were titer rises less than 4 fold for RS virus

Neutralizing (NT) antibodies

ECHO virus types 3 4 6 7 9 and 11 were used for neutralization with patient s sera in semi solid agar cultures of green monkey kidney (GMK) cells (27) A ≥10 fold titer increase was considered significant this corresponds to a ≥4 fold titer increase in conventional neutralization t sts

Virus isolation

Due to inadequate facilities for isolation of RS virus and parainfluenza virus these tests were omitted after a preliminary trial

RESULTS

The age distribution of the children is shown in Fig 1 Sixty one percent (27 patients) of the children wheezing for the first time were less than 1 year old and 89% (39 patients) were less than 2 years old The corresponding figures for the children who had had similar symptoms one or more times before were 36% (10 patients) and 75% (21 patients) respectively The ages of the six children who were older than 30 months were 33–66 months Of these children all except one (33 months old) had experienced wheezing before

The results of the virus serology studies may be seen in Fig 1 The two cases recorded below the zero line as possible RS virus infections showed a titer rise from <1/5 to 1/5–1/10

Atopic manifestations were noted in the past or present in 43% (9 of 21 cases) of parents and/or siblings among the children with high IgE while the corresponding frequency was 31% (16 of 51 cases) among the children with normal IgE Only 3 children had had atopic eczema before Two of these children were recidivating wheezers and had IgE levels of 441% and 885% of the mean for age The third child was a first time wheezer with an IgE level of 174% which did not exceed 2 S D of the mean for age

Blood eosinophilia (≥400/mm³) was noted in 67% (14/21) of cases with high IgE compared with 25% (13/51) of cases with normal IgE ($p < 0.002$) In most of the children with eosinophilia the number of eosinophils was low or normal in the acute phase and high in the convalescent phase In the group with high IgE eosinophilia was about as common among first time wheezers as among recidivating wheezers In the group with normal IgE however eosinophilia was more common among the recidivating wheezers but the difference was not significant More than 10 eosinophils in secretions was noted in only 2 of 48 children investigated in the acute phase Bronchial secretions were examined in one of the infants and nasal secretions in the other Both infants aged 7 and 11 months respectively were first time wheezers and had a normal blood eosinophil count The IgE levels were 369 and 454 respectively of the mean for age At the second examination 4 weeks later the total eosinophil counts were 950 mm³ and 733/mm³ respectively The IgE levels had then decreased to 329% and 406%

The age of onset of the first symptoms showed no significant difference between children with high and those with normal levels of IgE

DISCUSSION

The age distribution shows rather few infants in the age group of 0–6 months compared with other studies (12 17 37) This may depend on the fact that a real RS virus epidemic was not seen during the investigation period

The sex distribution in our study with 64% boys and 36 girls is in agreement with reported experiences of asthmatoid bronchitis (5 12 29 35) The same sex distribution is also typical for bronchial asthma

Several studies have shown the RS virus to be the most common virus in bronchiolitis and bronchopneumonia in infants (6, 28 37, 43) Frequencies between 15% and 75% have been noted in bronchiolitis and between 10% and 35% in bronchopneumonia During some epidemics even higher frequencies have been found (9 33 38) In our study 24% of the children showed titer rises against the RS virus but only ³/₄ of these titer rises were 4-fold or more However Ross et al (32) state that it may take 4–6 weeks especially in younger infants before maximal serological response is achieved In this study the mean period of time between the first and the second sample was 20 days It is therefore possible that some of the cases with a 2 fold titer rise from the first to the second sample might have risen to a significant level if the second sample had been taken later In the third serum sample from these cases the titers were again as low as in the first sample

Significant titer rises to parainfluenza virus were found in only 5 children Four had titer rises to type 3 and one to type 2 Parainfluenza viruses have certain antigens in common explaining why cross reactions may be seen between the different types (7) In our study no heterotypic antibody response was found but a definitive diagnosis requires virus isolation In addition to these cases titers (¹/ ₀–¹/₁₆₀) against type 3 were demonstrated in 9 acute sera indicating that this virus was prevalent in the population during that period The relationship between the childrens wheezing and a parainfluenza infection must in these cases be regarded as uncertain but cannot be excluded Both Schumacher (34) and Chanock et al (7) have reported parainfluenza virus infections with a late onset of wheezing. These studies suggest the possibility that some of our children with high initial titers had experienced a recent

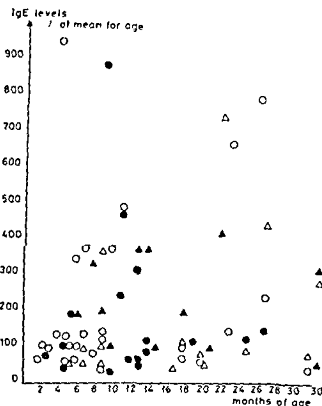


Fig 3 Relationship between virus serology findings and serum IgE levels in 72 infants with asthmatoid bronchitis ○ First time wheezers negative virus serology ● first time wheezers positive virus serology △ recidivating wheezers negative virus serology ▲, recidivating wheezers positive virus serology

but this difference was not pronounced Only 1 of the children with RS virus infection was severely ill

Pulmonary infiltrates were noted in 12 cases of which 4 had significant titer rises against RS virus, 2 against parainfluenza virus type 3 and one against ECHO virus type 9

Determinations of immunoglobulins G, A, M and D in the first 30 consecutive patients yielded grossly normal results In only 7 cases a moderate rise of immunoglobulin G was noted and in 4 cases a moderate rise of immunoglobulin A The immunoglobulin M levels did not in any case exceed 2 S D of the mean for age Because of these results no further determinations of these immunoglobulins were made

Determinations of immunoglobulin E showed *high levels (> 2 S D), corresponding to* 232–933% of the mean for age, in 21 cases Eleven of these children had wheezing for the first time (mean IgE 495% in acute phase, 496% in convalescent phase) while the other 10 children had had one or more similar attacks earlier (mean IgE 415% and 378% respectively) *Eleven of the children had a higher IgE* level in the first sample, the other 10 children in the second In 6 of the children the IgE level exceeded 2 S D of the mean for age only in the acute or in the convalescent serum

Positive virus serology was found to be as common among the children with high IgE as *among those with normal IgE* (Fig 3) Also RS virus infections were evenly distributed between children with high and normal IgE However the RS virus infected children wheezing for the first time had all normal IgE levels while 4 out of 5 of the RS virus infected children with former asthmatoid symptoms had high IgE levels (Table 2)

Table 2 *Asthmatoid bronchitis Relationship between immunoglobulin E levels virus serology and some clinical and laboratory findings prevalent in bronchial asthma The IgE levels are related to the upper 2 S D of the arithmetic mean value for age*

IgE levels	Children wheezing for the first time > 2 S D	< 2 S D	Children with recidivating wheezing > 2 S D	< 2 S D
Number of cases	11	33	10	18
Virus serology positive				
Total	3	14	6	8
RS	—	8	4	1
Allergic heredity in parents and/or siblings	5	12	4	4
Eosinophils ⩾400/mm³	7	6	7	7
Other atopic manifestations	—	1	2	—
Mean age of onset (months)	13 4	11 2	12 4	8 7

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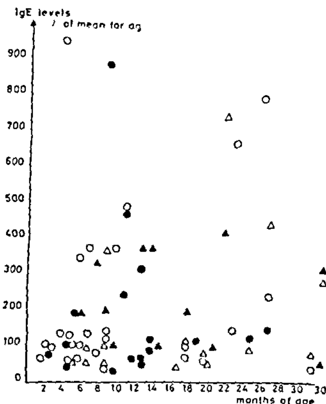


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Children with high IgE levels had a higher frequency of allergic heredity eosinophilia in blood or secretions and previous eczema. They were also older when they had their first wheezing episode However the differences were rarely statistically significant The same differences between children with high and normal IgE levels were found whether they were first time wheezers or not

To carry out allergological investigations in a conventional way with intracutaneous and provocation tests is very difficult in infants and small children The possibility of an in vitro diagnosis of atopic diseases would therefore be of great interest In spite of the limitations discussed above our impression is that the serum IgE level can be of help in assessing the possible atopic state of children with wheezing related to respiratory tract infections In conditions such as asthmatoid bronchitis with an eventual allergic component and where the possible allergens are unknown estimations of the serum IgE level is probably the best immunological screening method A high value should call for further allergological investigations A normal value on the other hand should lead to further investigations when there is a clinical suspicion of atopy The sera may then be tested in vitro for reaginic activity against common allergens in childhood (41) This procedure will probably facilitate further allergological investigations (4)

We intend to make repeated controls for some further years of the children in this study including a follow up of clinical histories determination of serum IgE levels and possible reaginic antibodies against some of the most common allergens In this way we hope to obtain a better understanding of the value of these immunological in vitro methods as diagnostic and prognostic adjuncts in asthmatoid bronchitis

SUMMARY

Seventy two children with wheezing related to respiratory tract infections have been investigated regarding virus serology and serum IgE levels Positive virus serology with the CF technique was achieved in 18% to RS virus in spite of absence of epidemics Seroconversion to ECHO viruses types 3, 6, 7 9 and 11 was seen in altogether 18% Antibody titers indicating infections with parainfluenzavirus adenovirus or influenza A 2 virus were found less frequently Twenty one of the infants had high IgE levels and these infants had allergic heredity recidivating symptoms former atopic manifestations and eosinophilia in blood and secretions more often than the infants with normal IgE Positive virus serology was found as often in the infants with high IgE as in those with normal IgE Determinations of the serum IgE levels seem to be of value in assessing the possible atopic component in children with asthmatoid bronchitis

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parainfluenza infection which had provoked their wheezing

Adenoviruses are held to be a rather common cause of bronchitis and pneumonia in infants On the other hand *these viruses seldom* cause bronchiolitis Hornsleth (18) found positive serology to adenovirus in 6–8% of pneumonia in children, while Chanock & Parrott (6) and Loda et al (28) found positive serology to this virus in only 2 4% and 1% respectively in children with bronchiolitis In 2 of our 3 children with titer rises to adenovirus there was a simultaneous titer rise against another virus The clinical significance of these adenovirus titers must therefore be regarded as uncertain

ECHO viruses as a cause of wheezing has not been frequently reported It is probably due to difficulties *in the serological diagnosis No* evidence of crossreaction with Herpes simplex virus was found in this study

During the winter 1968–69 there was an epidemic of influenza A-2 (Hongkong) in the population In spite of this there was only one child in our study with a titer rise against this virus

Other less common causes of wheezing in infants and children such as Mycoplasma pneumoniae and rhinovirus have not been investigated *This was because the principal aim* of the study was to get an understanding of the distribution of the most common virus types in asthmatoid bronchitis in relation to a possible atopy in the child

Infections with bronchiolotropic viruses are considered to give a swelling of the mucous membranes which along with the increased secretions cause obstructive bronchial symptoms The symptoms are accentuated *in infants* by the compression of their delicate and weak bronchial tree that accompanies the increased intrathoracic pressure during the forced expiration The view that bronchial obstruction occurring in virus infected children is merely of a non specific mechanical nature is held by Simon & Jordan (37) In a study of children with bronchiolitis, they concluded that children with a demonstrable RS virus infection *had a smaller risk* of developing bronchial asthma than those where such an infection could not be demonstrated

It is noteworthy, however, that RS virus *infected infants* so often develop severe respiratory tract infections despite the presence of *maternal neutralizing antibodies (8)* RS vaccinated infants often become sicker than non vaccinated infants when infected with RS virus (25) These data have led some authors to suspect an immunologic background to the symptoms in the children having antibodies to RS virus at the onset of a RS virus infection (8, 15) However, no definite relationship has been found in this study between the appearance of a certain virus type and an atopic constitution of the child

A high level of IgE in serum has been noted in about 60% of children with extrinsic asthma, *and in about* 30% of children with allergic rhino-conjunctivitis (3) Other diseases in which high IgE levels have been found are atopic eczema and certain parasitic diseases (24) In northern latitudes where parasitic diseases are rare, a high serum level of IgE probably indicates atopy A normal or low IgE level does not exclude an atopic disease *however If the* child has symptoms only during a certain *period of the year the* serum IgE *may* be increased during this time but return to the normal during the rest of the year (3) Even children with non seasonal extrinsic asthma may have normal IgE levels Different allergens seem to stimulate reagin production to a varying degree Dust and moulds in this respect seem to be weak allergens and this can perhaps explain why some asthmatic children have nor*mal IgE levels (3)*

In our study we found serum IgE levels exceeding 2 S D of the mean for age in about 30% (21 of 72 cases) These high IgE levels are probably an expression of atopy with a production of reaginic antibodies Evidence for atopy in these children compared to the children with normal or low IgE levels was the high frequency of such factors that are held to *be prognostically unfavourable* (Table 2)

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such as malnutrition malabsorption or skeletal, kidney or endocrine disorders Thus the material was regarded as normal with respect to the calcium distribution in plasma

METHOD

Principle of the method It is well known that pH and temperature influence the distribution of calcium between ionized and the protein bound fractions. Therefore these factors were controlled The ultrafiltrate was prepared by an over pressure and the Ca/K ratio of the ultrafiltrate was determined. The determination of the ultrafiltrable calcium (Ca_{e}) was made on the basis of the formula

$$Ca_{uf} = \frac{Ca_f \; K_p}{K_f}$$

in which Ca_{uf} = ultrafiltrable calcium in mg/100 ml plasma Ca_f = calcium in mg/100 ml ultrafiltrate K_p = potassium in mmol/l plasma K_f = potassium in mmol/l ultrafiltrate

Thus the method presupposes that the Ca/K ratio in the ultrafiltrate is equal to the ratio between ultrafiltrable calcium in plasma and plasma potassium

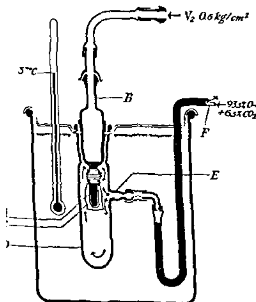


Fig 1 The ultrafiltration set *A* = collodion membrane sac *B* = adapter with a B 29 cone for tube *D* and an upper 18 19 socket ball. *C* = plastic bag loosely tied around the collodion membrane and with some small holes in its upper part. *D* = test tube with a side outlet = *E* *F* = plastic tubing inlet for the CO_2-O gas The black outer plastic tubing works as an outlet for the gas

Preparation of the samples Blood was drawn from fasting subjects and was taken at minor venous stasis (not exceeding 50 mmHg) and collected in heparin tubes Umbilical vein blood was drawn at birth within five minutes after birth Plasma was separated by centrifugation A small sample was used to determine the concentration of calcium and potassium The remaining sample was stored at +37 C

Ultrafiltration apparatus Ultrafiltration was performed in the apparatus shown in Fig 1 The apparatus was a modification of the one described by Rose (2.) A collodion shell (Sartorius membran filter GmbH No 13200 Membranfiltergesellschaft Gottingen West Germany) was shortened by cutting off its upper part and using the lowest 2.5 cm This sac (*A*) was attached to the funnel shaped lower part of the adapter (*B*) which was composed of a B 29 cone and an upper 18/19 socket ball (Quickfit) The sac was put into a plastic bag (*C*) which was about 3 cm long and which had a somewhat larger diameter than the collodion sac and a few small holes in its upper part The plastic bag was loosely tied around the collodion sac An 11×3 cm test tube (*D*) with a B 29 socket was attached to the adapter The test tube had a side outlet (*E*) which was lengthened with a rubber tubing through which a thin plastic tubing (*F*) was inserted and connected to a gas cylinder (93 5 O +6.5 CO) The apparatus was submerged into a water bath at +37 C as shown in Fig 1 After adding plasma as described below the socket ball of the adapter (*B*) was attached to a cone cup S 19 (Quickfit) connected to a nitrogen cylinder and a pressure gauge Between runs the collodion sac was tored in saline

Procedure 1 5–2 ml plasma was transferred to the collodion sac (*A*) The CO_2-O mixture was supplied through the thin plastic tubing (*F*) After temperature equilibration to +37 C the adapter (*B*) was connected to the nitrogen cylinder and a pressure of 0 6 kg/cm was applied

An ultrafiltrate soon appeared in the plastic bag (*C*) in contact with the collodion sac (*A*) 0 5 ml required about 10–15 min and was sufficient for duplicate determinations of calcium and potassium at the same dilution (1 20) in an Eppendorf flame photometer The plasma residue in the collodion sac (*A*) was used for pH determination with a Radiometer capillary electrode type G 297/92 (accuracy ±0 01 pH unit at constant temperature)

COMMENTS ON THE METHOD

Measurements of pH before and after the ultrafiltration procedure showed a very small mean change of ±0 01 units when the CO–O mixture was applied continuously *It is thus sufficient* to determine the pH of the residue in the collodion sac after ultrafiltration as described in the procedure

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PLASMA CALCIUM FRACTIONS IN NORMAL SUBJECTS FROM BIRTH TO ADULT AGES

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Total calcium in blood plasma appears in three different fractions ionized protein bound and complexed (as citrate, phosphate sulphate and carbonate) The ionized calcium is kept within narrow limits by the regulatory function of the parathyroid glands and of the hormone thyreocalcitonin from the parafollicular cells in the thyroid gland Complexed calcium is a small fraction and has not been subjected to intensive study Ultrafiltrable calcium comprises ionized and complexed calcium

The amount of calcium bound to the plasma proteins is said to be determined primarily by the concentrations of ionized calcium and plasma proteins Thus, there are those who maintain that the determination of ionized calcium or ultrafiltrable calcium adds nothing to the information gained from the determination of the total plasma calcium, at least not in patients with endocrine disorders (14) Other authors however are of the opinion that different protein fractions have different capacities to bind calcium (8 10) Furthermore it has been pointed out that ultrafiltrable and protein bound calcium may deviate from the normal distribution under different clinical conditions (6, 25) Bronner (2) concluded that our knowledge in this field is incomplete Consequently he preferred direct measurement of ultrafiltrable calcium to calculation on the basis of the nomogram of McLean & Hastings (15) which is based on the total calcium and the total plasma protein concentrations

Numerous reports on the different calcium fractions in plasma of adults have been published So far however we have found only one report (24) on the normal distribution of the calcium fractions in plasma from children of different ages Therefore the aim of the present investigation was to study the changes of the calcium fractions from birth up to adult ages A simple and rapid method to determine ultrafiltrable calcium in plasma was developed for this purpose and will be described

MATERIAL

The material comprised 202 children aged 0–16 years (for age distribution see Figs 3 and 4) and 93 healthy male blood donors aged 18 62 years The material was divided into age groups as shown in Figs 3 and 4 Sixty six children were studied during their first week of life in a maternity ward Delivery of all the infants was spontaneous vaginal and without complication None of them suffered asphyxia and the neonatal periods were uneventful They were all breast fed beginning after 24 hours of life Of the 14 children from 4 months up to one year seven were patients at an orphanage The remaining 129 subjects had been admitted to the Children's Hospital of Göteborg for minor disorders usually phimosis retentio testis or inguinal hernia No child had a disorder that could be expected to disturb the calcium phosphorus or protein metabolism

value for which the limits are sought and *s* the variation around the regression As the calcu ations are based on the assumption that there is a linear relationship within the inter vals chosen and as the error inherent in the estimation of the independent variate is neglected the fiducial limits obtained are only approximate The present results indicate that pH has its greatest effect on the distribution of plasma calcium within the pH range com patible with life In that range our curve was much steeper than the ones reported earlier (9 13)

The physiological range for total plasma calcium concentration is known to be narrow hence the range of ultrafiltrable calcium should be even more narrow However if the pH is not taken into full account the standard devia tion for ultrafiltrable calcium concentration in plasma will be more than twice that of total plasma calcium When the pH is taken fully into account the standard deviation for ultra filtrable calcium will be reduced to the same magnitude (±0 23 mg/100 ml) as that for total plasma calcium Thus it is of utmost importance to know plasma pH and keep it constant during the ultrafiltration For the sake of comparison it might be better to recal culate all values to one and the same pH say 7 40 by consulting the curve in Fig 2

The error (coefficient of variation) for Ca_p and K_p was 0 8 ($n = 93$) The error (coef ficient of variation) for the Ca_f determina tion was calculated on the basis of duplicate de erminations and was found to be 1 5 when two different membranes were used ($n = 25$) and 1 3 when one membrane was used ($n = 173$)

RESULTS

Figs. 3 and 4 show the distribution of plasma calcium in the different age groups No sig nificant differences were found between males and females in childhood Therefore mean values and standard deviations for total cal cium ultrafiltrable calcium and protein bound calcium are given without regard to sex

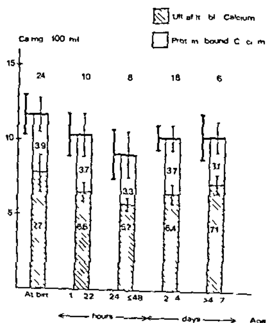


Fig 3 Distribution of plasma calcium during the first week of life Sixtysix normal cases Mean values and standard deviations for total calcium (thick bars) ultrafiltrable calcium and protein bound calcium are given All values corrected to pH 7 4

Total plasma calcium showed a significantly higher mean value in umbilical cord blood (11 6 mg/100 ml) than in all other age groups ($p < 0 01$) At 24–48 hours of life there was a decrease to a mean of 9 0 mg/100 ml Later an increase in the total calcium was seen up to three months of age ($p < 0 01$) The mean values found between three months and up to one year of age were significantly higher than those seen after six years of age ($p < 0 01$) All age groups up to 16 years except for the group 24–48 hours showed significantly higher total plasma calcium levels than adults ($p < 0 01$)

Ultrafiltrable calcium (recalculated to pH 7 4) in umbilical cord blood plasma was sig nificantly higher (mean 7 7 mg/100 ml) than in all other age groups ($p < 0 01$) After birth the e was a decrease of the Ca_f fraction which reached its minimum value at 24–48 hours after birth (5 7 mg/100 ml) The mean value at 1–21 h of life was significantly higher ($p < 0 01$) than the mean value at 24–48 h An

In previous methods, ultrafiltrable calcium has been determined on the basis of a direct analysis of calcium in the ultrafiltrate

Transformation of the calcium concentration of the ultrafiltrate into the plasma concentration requires a correction for plasma protein volume and possibly also for the Donnan effect The latter correction may not be reliable as the pressure applied during ultra filtration does not lead to equilibrium which is one prerequisite for the Donnan membrane theory With the present method no consideration has to be taken to any of these corrections as the calculation is based on a ratio between the two components in the ultrafiltrate (Ca/K) Moreover, no consideration has to be taken to possible dilution caused by water remaining in the pores of the collodion sac, nor to inaccurate dilution of the sample for the flame photometric determination

The prerequisite for the present method is that no part of the plasma potassium is bound to plasma protein Our results are in agreement with the view that all plasma potassium is ultrafiltrable In carefully handled samples from our normal material, the potassium concentration in the ultrafiltrate corresponded well with the potassium concentration in plasma $K_f = 0\,96\ K_p + 0\,11$ Moreover if a part of the plasma potassium were protein bound our calculation procedure should give erroneously high values for Ca_{uf} Our values for adults however, agree with those given in recent reports (1, 13, 18, 21) and are below those of older studies (9, 19 22, 27)

The relationship between pH and ultrafiltrable calcium for the 93 healthy adult blood donors is shown in Fig 2 Each dot represents the mean of two determinations on the same ultrafiltrate In order to study the relation between pH and Ca_{uf} outside the physiological pH range 0 05–0 20 ml M HCl or M NaOH was added to 15 ml plasma (80 samples)

Fig 2 shows the fiducial intervals around the regression line This line embraces the area within which 95% of individual values for the pH range 7 1–7 6 are expected to fall (4) The calculation was made according to the formula

$$y = \pm t\sqrt{s^2\left(1 + \frac{1}{n} + \frac{(x - \bar{x})^2}{\sum(x - \bar{x})^2}\right)}$$

in which t is the 0 05 probability deviate with n 1 degrees of freedom x is any particular

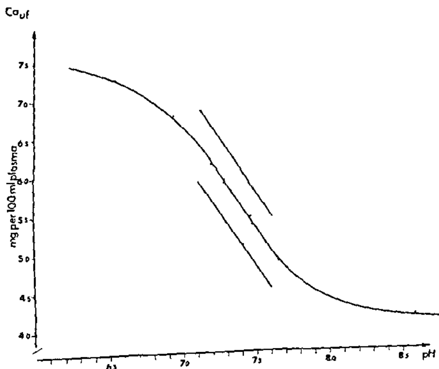


Fig 2 Ultrafiltrable calcium in mg 100 ml plasma at different pH and +37 C Ninety three normal cases The pH values outside the physiological interval were obtained by adding HCl and NaOH

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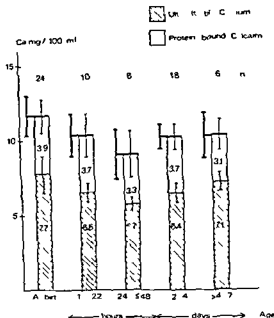


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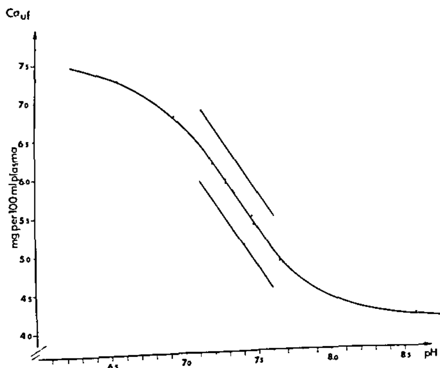


Fig 2 Ultrafiltrable calcium in mg 100 ml plasma at different pH and +37 C Ninety three normal cases The pH values outside the physiological interval were obtained by adding HCl and NaOH

Our mean value for umbilical vein blood is within the ranges given by these authors Our mean value for Ca $_f$ in umbilical vein blood plasma is equal to that given earlier in the literature (5) Teh (24) found ultrafiltrable calcium to decrease with age from 65 % of total calcium to 55 5 in adult ages during the first four weeks of life The significant decrease in ultrafiltrable calcium seen in our material from the first week of life seems to be responsible for the entire decrease in total plasma calcium However during the first week of life there is a decrease in protein bound calcium possibly because of the lowered amount of circulating plasma albumin (16 17)

Thus it can be concluded that the higher total plasma calcium levels seen during childhood are caused by an increased ultrafiltrable calcium fraction It seems reasonable to assume that this in turn indicates a positive calcium balance and skeletal mineralization

SUMMARY

A simple and rapid method to determine ultrafiltrable calcium in plasma is described in detail Total calcium ultrafiltrable calcium (Ca $_f$) and protein bound calcium in plasma are reported for 202 normal children (range 0–16 years of age) divided into 12 age groups as well as for 93 healthy male blood donors There was a decrease in plasma calcium and ultrafiltrable calcium after 1–21 hours of life The minimum value was reached at 24–48 hours of life An increase was then seen during the first week of life Later in childhood total calcium and Ca $_f$ decreased continuously and significantly with age and with the most marked rate during the first two–three years

The ultrafiltrable calcium fraction was found to be responsible for the changes in total plasma calcium In 24–48 hours of life and 5–7 days of life protein bound calcium was significantly lower as well There were no significant differences between males and females in childhood

ACKNOWLEDGEMENTS

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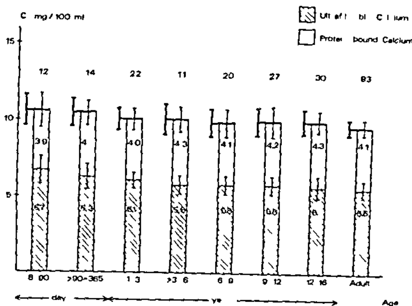


Fig 4 Distribution of plasma calcium after the first week of life up to adults 229 normal cases Mean values and standard deviations for total calcium (thick bars) ultrafiltrable calcium and protein bound calcium are given All values corrected to pH 7 4

increase was then seen with significant difference between the 2–4 day group and the 24–48 h group ($p < 0.01$) as well as between the 4–7 day group and the 2–4 day group ($p < 0.01$) Up to three years of age, the ultrafiltrable calcium fractions were significantly higher than in the 3–6 year group ($p < 0.05$) and later in life ($p < 0.01$) Up to twelve years of age, the level was still significantly higher than in the adults ($p < 0.01$)

Protein bound calcium showed its significantly lowest value at 24–48 hours of life and at the end of the first week of life ($p < 0.01$) Later there were some significant differences between the groups, however, without any certain tendency

DISCUSSION

The technique used for blood sampling varied with the age group No venous stasis was applied before three years of age At birth blood was drawn from vena umbilicalis Up to one year of age blood was drawn from vena jugularis interna From one year to three years of age, the blood was drawn from vena femoralis, and after three years from the cubital vein Venous stasis above a certain pressure is known to induce an in vivo ultrafiltration which should mean a rise in total plasma calcium (12) The venous stasis used by us was below the critical level During the first month of life, the meals are so frequent that a true fasting state does not exist This can partly account for the increased plasma calcium levels in these ages The number of meals then decreases to five per day and is then further reduced to four a day at four months of age The significance of this is difficult to determine

Total plasma calcium has been reported to be higher in children than in adults already shown in 1921 (11) Later reports on this subject however are rare and only one (24) reports a variation in total plasma calcium and Ca_{uf} with age According to Teh (24) only blood collected at birth contains significantly higher amounts of total calcium than the blood in adults During the first week of life, the level was significantly lower than in adult life All of our values are higher than those of Teh (24) and thus more in conformity with the older concept The decrease in total plasma calcium during the first week of life has been reported earlier (3 20 23) The minimum level found by us is in agreement with recent reports (7 28) Several investigators (for references, see 5 26) have found that total plasma calcium in umbilical cord is higher than in maternal plasma

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METABOLIC ALKALOSIS IN INFANTS ROLE OF WATER DEPLETION AND CHANGES IN COMPOSITION OF STOOL

Review of a Physiological Problem

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the pathophysiologic interpretation of any :tabolic alkalosis two obligatory require nts must be met firstly the induction of disturbance must be accounted for by iden ying a quantitatively meaningful net gain of ratable base secondly the maintenance of e disturbance must be accounted for by iden ying the factor(s) instrumental in the asso ated rise in the renal bicarbonate threshold the rise in the rate of generation of bicarbo e by the renal tubular epithelium

In recent years a number of determinants th rate of tubular bicarbonate generation ve been studied under both experimental and nical conditions These include the arterial co, mineralocorticoid hormones and stores potassium and chloride and it appears that reduced rate of glomerular filtration of chlo- de is an important factor in the maintenance gastric alkalosis (4) On the other hand nited attention has been paid to the question the source of the priming net load of base he present paper purports to examine this oblem in the light of new developments in e area of gastrointestinal acid base physiology pecifically the role of gastric hydrochloric id depletion will be reconsidered in the light f obligatory effects of concomitant changes in e balance of water

upported by grant no 512-606 from the Danish ate Medical Research Council

It is generally held that in prolonged obstructive vomiting depletion of gastric hydrochloric acid is responsible for the induction of the alkalotic disturbance This view is simple and fits the Bronsted formulation of acid base chemistry well It will be observed however that sustained losses of hydrochloric acid from the stomach (which do occur in pyloric stenosis) do not necessarily imply net losses of acid from the body a point which has attracted few acid base physiologists Similarly although the phenomena of dilution acidosis and concentration alkalosis have received some attention (1 13) the implications of the underlying principle for our understanding of several common clinical acid base disturbances especially post vomiting alkalosis appear to have been overlooked

Open and closed titration systems the titratable value of water

Acid base titration involves evaluation of concentrations of acid or base in terms of quantities of hydroxyl ion or hydrogen ion added per unit of sample volume in order to produce a specificed change in pH Titrating bicarbonate containing buffer solutions like the body fluids requires additional specifications of any change in P_{CO} and total CO_2 concentration associated with the titration Titration at a constant total CO_2 concentration (closed system titration) is

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METABOLIC ALKALOSIS IN INFANTS ROLE OF WATER DEPLETION AND CHANGES IN COMPOSITION OF STOOL

Review of a Physiological Problem

POUL KILDEBERG and KNUD ENGEL

From the Departments of Paediatrics Clinical Chemistry and Physiology Odense University Medical School Odense Denmark

In the pathophysiologic interpretation of any metabolic alkalosis two obligatory requirements must be met firstly the induction of the disturbance must be accounted for by identifying a quantitatively meaningful net gain of titratable base secondly the maintenance of the disturbance must be accounted for by identifying the factor(s) instrumental in the associated rise in the renal bicarbonate threshold i e the rise in the rate of generation of bicarbonate by the renal tubular epithelium

In recent years a number of determinants of the rate of tubular bicarbonate generation have been studied under both experimental and clinical conditions These include the arterial P_{CO_2} mineralocorticoid hormones and stores of potassium and chloride and it appears that a reduced rate of glomerular filtration of chloride is an important factor in the maintenance of gastric alkalosis (4) On the other hand limited attention has been paid to the question of the source of the priming net load of base The present paper purports to examine this problem in the light of new developments in the area of gastrointestinal acid base physiology Specifically the role of gastric hydrochloric acid depletion will be reconsidered in the light of obligatory effects of concomitant changes in the balance of water

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It is generally held that in prolonged obstructive vomiting depletion of gastric hydrochloric acid is responsible for the induction of the alkalotic disturbance This view is simple and fits the Bronsted formulation of acid base chemistry well It will be observed however that sustained losses of hydrochloric acid from the stomach (which do occur in pyloric stenosis) do not necessarily imply net losses of acid from the body a point which has attracted few acid base physiologists Similarly although the phenomena of dilution acidosis and concentration alkalosis have received some attention (1 13) the implications of the underlying principle for our understanding of several common clinical acid base disturbances especially post vomiting alkalosis appear to have been overlooked

Open and closed titration systems the titratable value of water

Acid base titration involves evaluation of concentrations of acid or base in terms of quantities of hydroxyl ion or hydrogen ion added per unit of sample volume in order to produce a specificed change in pH Titrating bicarbonate containing buffer solutions like the body fluids requires additional specifications of any change in P_{CO_2} and total CO_2 concentration associated with the titration Titration at a constant total CO concentration (closed system titration) is

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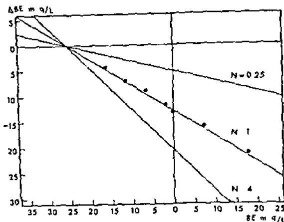


Fig 1 Changes in blood base excess" (ΔBE) resulting from the addition of water as a function of the initial blood BE Solid lines represent equation (v) for various values of *N* Dots represent results of mixing experiments for $N = 1$ using 0 9% sodium chloride as the diluent

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That the order of magnitude of the dilutional effect of water on the blood BE demonstrated in Fig 1 may apply to in vivo alkalosis can be realized from the following hypothetical examples

Consider a four kilogram infant with 350 ml/kg of extracellular fluid (3) Following prolonged obstructive vomiting the water deficit may reach 15% of the body weight (1?) i e 600 ml and the blood BE may rise to + 0 mEq l (5) Assuming the dehydration to be iso-osmolar (5 1_) the contraction will be confined largely to the extracellular space Assuming further that the BE of interstitial fluid is roughly the same as that of blood (allowing for in vivo bicarbonate redistribution) 800 ml of extracellular fluid will be left containing 0 8 × 0 = 16 milliequivalents of titratable base Upon subsequent restoration of a normal extracellular fluid volume by infusion of 600 ml of isotonic bicarbonate free solution representing 0 6 × 25 5 = 15 3 milliequivalents of titratable acid the 1 400 ml of extracellular fluid will now contain only 0 7 milliequivalents of titratable base which means that the blood BE will have fallen to + 0 5 mEq l

In the case of mild dehydration such an infant may typically lose 50 ml/kg body weight of extracellular fluid and the blood BE may be + 6 0 mEq l Under such circumstances the calculated effect of rehydration will again suffice to restore a normal blood BE (+1 5 mEq l)

Thus to the extent that simple rehydration suffices to correct the alkalosis the latter can be conceived as a mere consequence of extracellular fluid contraction Obviously such considerations do not permit of the conclusion that a negative water balance is the sole primary cause of elevation of the BE of blood in obstructive vomiting They do in our opinion suffice to establish water loss as one major determinant as well as to point to the need for a closer view on the relation between gastric HCl depletion and the net input of base from the gastrointestinal tract The latter can be studied conveniently in terms of a titrational end point at pH 7 40 and P_{CO} zero mmHg

Vomiting and gastrointestinal acid base exchange

The parietal cells of the gastric glands generate hydrogen ions These are secreted into the lumen at the expense of the equivalent quantity of hydroxyl ion being added (as bicarbonate) to the venous return Similarly the pancreatic acini secrete bicarbonate at a rate equivalent to the rate of acidification of the pancreatic venous blood The jejunal glands like the gastric epithelium produce an acid juice charging the blood with base at the equivalent rate (8 11) whereas finally the ileal and colonic secretions are alkaline (9) In so far as direct ionic

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Consider an X millimolar solution of NaOH titrated with HCl to pH 7 40 Representing the initial pH by x the result of simple titration at Pco zero mmHg may be stated as follows (TA denoting titratable acid)

$$[TA]_{pH\ x\ Pco\ 0}^{pH\ 7\ 40\ Pco\ 0} = -X\,mEq/l \qquad (i)$$

Titration of the same NaOH solution to pH 7 40 and Pco 40 mmHg however involves CO_2 absorption and the generation of bicarbonate $CO_2 + OH^- \rightarrow HCO_3^-$ Taking the pK_A of carbonic acid and the CO_2 absorption coefficient to be 6 10 and 0 032 respectively for pure water at body temperature (10) the Henderson-Hasselbalch equation shows that 25 5 mmol/l of bicarbonate have been formed when the end point is reached

$$7\ 40 = 6\ 10 + \log\frac{25\ 53}{0\ 032 \times 40}$$

Consequently only $X - 25\ 5$ mmol/l of HCl will be required to reach pH 7 40 the remaining hydroxyl ions being titrated with CO

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In a phosphate bicarbonate buffer in equilibrium at pH 7 40 and Pco 40 mmHg gain of Z mmol/l of NaOH at an unchanged Pco will lead to CO_2 absorption Upon subsequent titration with HCl the same quantity of CO_2 will be released hence

$$[TA]_{pH\ z\ Pco\ 40}^{pH\ 7\ 40\ Pco\ 40} = -Z\,mEq/l \qquad (iii)$$

where z represents the sample pH following gain of Z mmol/l of NaOH For closed system titrations an expression analogous to (i) and (iii) obtains

In general for any acid base system a family of parallel TA scales exist for each end point pH These scales conform to the general equation $TA = n - X$ where X denotes milliequivalents of base accumulated including any bicarbonate The significance of n becomes clear when X assumes the value of zero in which case $TA = n$ Thus n is the titratable value of the solvent i e water For example titrating water from pH 7 40 (assuming a trace of NaOH to be present initially) at Pco zero mmHg to pH 7 40 at Pco 40 mmHg involves the addition of 25.5 mmol of NaOH to each liter of water

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We may now rewrite equation (iii) in these terms

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In this formulation equation (iii) represents the "base excess (BE) scale of blood cf (10)

It follows that with respect to the particular end point considered pH 7 40 at P_{CO} 40 mmHg water must be regarded an acid, albeit in molar terms an exceedingly weak one Whereas one mole of HCl represents one mole of titratable hydrogen ion (i e , one equivalent), one mole of water (18 ml) represents only $0\ 0255 \times 10^{-3} \times 18 = 0\ 00046$ moles of titratable hydrogen ion Furthermore, whereas the volume effect of strong acid or base per se accumulating within the limits of tolerance of the body is negligible, the volume effect of water is substantial Hence *the amount of strong acid or base* gained in conditions of a zero water balance is directly related to the resulting change in the concentration of TA in the body fluids whereas the effect of a given water load depends on the pre existing level of the latter Provided the TA of the body fluids is less than 25 5 mEq/l, water accumulation will cause the TA to rise If however, the body fluid TA is above 25 5 mEq/l (extreme *metabolic acidosis*) a water load will cause the TA to fall A quantitative formulation of this effect can be derived from a few numerical examples

Mixing equal parts of normal blood (TA = − BE = zero mEq/l) and isotonic sodium chloride solution *(TA = 25 5 mEq/l) results in* a change in the blood BE from zero to − 12 75 mEq/l Mixing one part of blood with a BE of + 25 5 mEq/l and two parts of isotonic saline produces a fall in blood BE from + 25 5 to − 8 5 mEq/l etc Hence the calculated effect of the initial blood BE on *the change* in blood BE resulting from mixing blood and water (e g as 0 9% NaCl) can be formulated as follows

$$\Delta BE = -\frac{N}{N+1}(25\ 5 + BE_{initial}) \qquad (v)$$

where N is the number of parts of water added to one part of blood Clearly the effect is small for

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In chemistry acid base titrations are carried out to an arbitrary end point at pH 7 00 (temperature 25°C P_{CO} zero mmHg) the advantage being that solutes can be titrated with negligible interference by the aqeous medium Thus the conventional acid base diagnostic system using average normal (adult) blood acid base values as the arbitrary basis for statements concerning titratable values may seem unnecessarily complicated It does however constitute a frame of reference within which the effects on the pH of the body fluids of exchanges of acid (base) and water can, and should, be explored separately

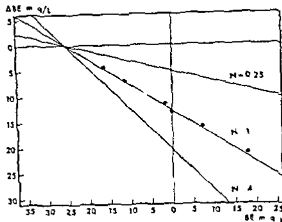


Fig 1 Changes in blood "base excess" (ΔBE) resulting from the addition of water as a function of the initial blood BE. Solid lines represent equation (v) for various values of N Dots represent results of mixing experiments for N=1 using 0.9% sodium chloride as the diluent

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That the order of magnitude of the "dilutional effect of water on the blood BE demonstrated in Fig 1 may apply to in vivo alkalosis can be realized from the following hypothetical examples

Consider a four kilogram infant with 350 ml kg of extracellular fluid (3) Following prolonged obstructive vomiting the water deficit may reach 15% of the body weight (1?) i e 600 ml and the blood BE may rise to + 0 mEq l (5) Assuming the dehydration to be iso-osmolar (5 1) the contraction will be confined largely to the extracellular space Assuming further that the BE of interstitial fluid is roughly the same as that of blood (allowing for in vivo bicarbonate redistribution) 800 ml of extracellular fluid will be left containing 0 8 ?0 = 16 milliequivalents of titratable base Upon subsequent restoration of a normal extracellular fluid volume by infusion of 600 ml of isotonic bicarbonate free solution representing 0 6 × 25.5 = 15 3 milliequivalents of titratable acid the 1 400 ml of extracellular fluid will now contain only 0 7 milliequivalents of titratable base which means that the blood BE will have fallen to +0.5 mEq/l.

In the case of mild dehydration such an infant may typically lose 50 ml/kg body weight of extracellular fluid and the blood BE may be +6 0 mEq l. Under such circumstances the calculated effect of rehydration will again suffice to restore a normal blood BE (+1.5 mEq/l)

Thus to the extent that simple rehydration suffices to correct the alkalosis the latter can be conceived as a mere consequence of extracellular fluid contraction Obviously such considerations do not permit of the conclusion that a negative water balance is the sole primary cause of elevation of the BE of blood in obstructive vomiting They do in our opinion suffice to establish water loss as one major determinant as well as to point to the need for a closer view on the relation between gastric HCl depletion and the net input of base from the gastrointestinal tract The latter can be studied conveniently in terms of a titrational end point at pH 7 40 and P_{CO_2} zero mmHg

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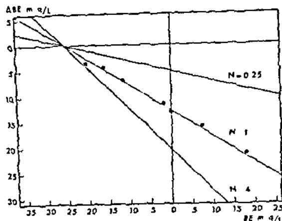


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where N is the number of parts of water added to one part of blood Clearly the effect is small for

heavily acidotic blood but very appreciable for samples with BE levels in the alkalotic range Results of actual mixing experiments for $N=1$ are presented in Fig 1

In chemistry acid base titrations are carried out to an arbitrary end point at pH 7 00 (temperature 25 C P_{CO} zero mmHg) the advantage being that solutes can be titrated with negligible interference by the aqeous medium Thus the conventional acid base diagnostic system using average normal (adult) blood acid base values as the arbitrary basis for statements concerning titratable values may seem unnecessarily complicated It does however constitute a frame of reference within which the effects on the pH of the body fluids of exchanges of acid (base) and water can and should be *explored separately*

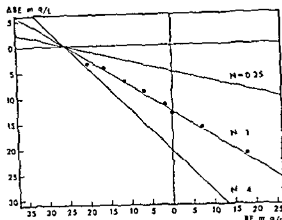


Fig 1 Changes in blood base excess" (ΔBE) resulting from the addition of water as a function of the initial blood BE Solid lines represent equation (v) for various values of *N* Dots represent results of mixing experiments for $N=1$ using 0 9% sodium chloride as the diluent

Implications for blood acid base status in obstructive vomiting

That the order of magnitude of the dilutional effect of water on the blood BE demonstrated in *Fig 1* may apply to in vivo alkalosis can be realized from the following hypothetical examples

Consider a four kilogram infant with 350 ml/kg of extracellular fluid (3) Following prolonged obstructive vomiting the water deficit may reach 15 % of the body weight (1) i e 600 ml and the blood BE may rise to +20 mEq/l (5) Assuming the dehydration to be iso-osmolar (5 17) the contraction will be confined largely to the extracellular space Assuming further that the BE of interstitial fluid is roughly the same as that of blood (allowing for in vivo bicarbonate redistribution) 800 ml of extracellular fluid will be left containing $0\,8\times 20=16$ milliequivalents of titratable base Upon subsequent restoration of a normal extracellular fluid volume by infusion of 600 ml of isotonic bicarbonate free solution representing $0\,6\times 25\,5=15\,3$ milliequivalents of titratable acid the 1 400 ml of extracellular fluid will now contain only 0 7 milliequivalents of titratable base which means that the blood BE will have fallen to +0 5 mEq/l

In the case of mild dehydration such an infant may typically lose 50 ml/kg body weight of extracellular fluid and the blood BE may be +6 0 mEq/l Under such circumstances the calculated effect of rehydration will again suffice to restore a normal blood BE (+1 5 mEq l)

Thus to the extent that simple rehydration suffices to correct the alkalosis the latter can be conceived as a mere consequence of extracellular fluid contraction Obviously such considerations do not permit of the conclusion that a negative water balance is the sole primary cause of elevation of the BE of blood in obstructive vomiting They do in our opinion suffice to establish water loss as one major determinant as well as to point to the need for a closer view on the relation between gastric HCl depletion and the net input of base from the gastrointestinal tract The latter can be studied conveniently in terms of a titrational end point at pH 7 40 and P_{CO} zero mmHg

Vomiting and gastrointestinal acid base exchange

The parietal cells of the gastric glands generate hydrogen ions These are secreted into the lumen at the expense of the equivalent quantity of hydroxyl ion being added (as bicarbonate) to the venous return Similarly the pancreatic acini secrete bicarbonate at a rate equivalent to the rate of acidification of the pancreatic venous blood The jejunal glands like the gastric epithelium produce an acid juice charging the blood with base at the equivalent rate (8 11) whereas finally the ileal and colonic secretions are alkaline (9) In so far as direct ionic

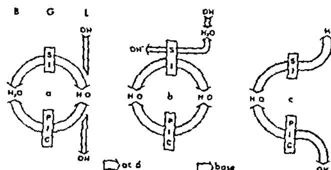


Fig 2 Suggested types of gastrointestinal acid base response *a* oral ingestion of base net zero absorption *b* oral ingestion of base net positive absorption *c* obstructive vomiting net zero absorption *S J P I C* Gastric jejunal pancreatic ileal and colonic glands respectively *B* Portal blood *G* Gastrointestinal membrane *L* Lumen

transport, from lumen to blood, plays only a *minor role in gastrointestinal* absorption of acid or base this serial glandular arrangement constitutes an almost perfect homeostatic system if the total secretion of hydrogen ion (by stomach and jejunum) and the total secretion of base (by pancreas and ileum colon) are maintained at *equal rates* there can be no net acidification of the portal blood ingested non-metabolizable acid or base being "shunted through the gastrointestinal tract to appear in the stool (7) On the other hand, net absorption of any given quantity of base during any given period of time requires the total amount of acid secreted during that period to exceed the amount of base concurrently secreted at other levels by the *identical* quantity, etc (Fig 2)

Thus according to present views on the mechanics of gastric hydrochloric acid production the immediate influence on the blood acid-base status is manifest at the moment of H^+ secretion and independent of the luminal fate of the generated hydrogen ions If in the event of vomiting, excess (not neutralized) pancreatic bicarbonate is left in the lumen the development of alkalosis is contingent upon reabsorption of this amount of base To the extent that the excess base is not reabsorbed (but lost with the stool) the blood acid base status will remain unaffected But reabsorption can occur only as a result of equivalent H^+ secretion in casu presumably by the jejunum Alternatively suppression of the rate of pancreatic bicarbonate secretion may take place as a direct consequence of gastric losses of hydrochloric acid Either way we are forced to accept the conclusion that in terms of gastrointestinal acid base physiology *a positive net base balance as an immediate effect of vomiting can be conceived only as a result of net hypersecretion of acid* Moreover, any homeostatic measure minimizing the rate of accumulation of base in the extracellular fluid must aim at maintaining identity between rates of secretion of acid and base at various levels of the gastrointestinal tract

That such an acid base homeostatic system *is indeed operative* in the gastrointestinal tract was suggested by previous studies of the balance of net acid (defined as non-carbonic, non metabolizable acid on titration to pH 7 40 and P_{CO_2} zero mmHg) in healthy infants (7) In these studies, an extremely close correlation was established between rates of dietary intake of net base (NB) represented by organic anion and rates of concurrent fecal net base excretion ($NB_{stool} = 0\,737 \times NB_{diet} + 1\,711$, $p < 0\,0005$) Further studies involving changes in the composition of the diet complemented these findings (2, 6) Once more, the effect of a change in the rate of oral ingestion of base was obviated by an almost equivalent change in the rate of stool losses of base Similar data on adults are not available Conceivably the gastrointestinal tract may respond in a similar fashion to losses of hydrochloric acid from the stomach the portal venous return escaping alkalinization (Fig. 2) Indeed, the gastrointestinal tract seems eminently suited to compensate for variations in the amount of acid or base offered for transport It is not suited to compensate for losses of water

SUMMARY

Simple calculations of the effect of volume depletion on the concentration of titratable base of blood show this effect to be largely adequate

to account for commonly observed degrees of metabolic alkalosis in patients with mild or extreme dehydration due to obstructive vomiting. This suggests that hydrochloric acid depletion per se may not be a major determinant of the blood acid base status in such conditions. Clearly part of the base equivalents missing in the extracellular fluid may be sought in the cell water but currently available evidence concerning gastrointestinal transport of acid and base invites a conception of gastrointestinal acid base *homeostasis*—and the hypothesis that in obstructive vomiting changes in the composition of stool may appreciably modify the effect of gastric hydrochloric acid depletion. In studies of intracellular buffering and net base balance in gastric alkalosis it must be realized that the primary extracellular disturbance may be largely contractional and that stool losses (or luminal pooling) of base may be an important variable.

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CONCENTRATION OF PURINE NUCLEOTIDES IN ERYTHROCYTES OF PATIENTS WITH THE LESCH-NYHAN SYNDROME BEFORE AND DURING ORAL ADMINISTRATION OF ADENINE

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Previously we reported the influence of oral adenine administration on the clinical features and purine metabolism in the Lesch-Nyhan syndrome (20) This syndrome is an X-linked recessive disorder with choreoathetosis, hyperuricaemia, mental retardation, aggressive behaviour compulsive automutilation and occasionally megaloblastic anaemia (9 11 12 14, 15) The syndrome is biochemically characterized by a deficiency of the enzyme hypoxanthine guanine phosphoribosyltransferase (HGPRT ase) EC 2 4 2 8 (17), and a greatly increased purine synthesis *de novo* (11, 13)

In view of the key position of the purine nucleotides in purine metabolism (Fig 1) it seemed worthwhile to determine the content of these nucleotides in erythrocytes of two patients with the Lesch Nyhan syndrome before and during oral administration of adenine

MATERIAL AND METHODS

Purine nucleotides were analysed according to a modified method of Hurlebert et al (8) The erythrocytes of 20 ml blood were haemolysed with one volume distilled water To the obtained haemolysate 20 ml of an ice cooled trichloric acetic acid (TCA) solution (10%) were immediately added This mixture was stirred vigorously and then centrifuged for 10 min at 3 000 r p m The obtained supernatant was kept in an ice water bath The precipitate was extract d twice with 20 ml of an ice-cooled TCA solution (5) and centrifuged as given before The combined supernatants were extracted 6 times with equal volumes of diethylaether to remove the TCA The resulting aqueous solution with a pH of about 6 was applied to a Dowex 1 × 2 (200–400 mesh) column (25 × 0 7 cm) equilibrated with 0 8 M ammoniumformate in 6 N formic acid Elution was performed with two consecutive linear gradients I distilled water to 4 N formic acid (total volume 800 ml) and II 4 N formic acid to 0 8 M ammoniumformate in 4 N formic acid (total volume 800 ml) Fractions of 5 ml were collected

The absorbance of the eluate was continuously registered at 254 nm during the elution The obtained peaks were identified by measurement of the absorbance at 260 265 270 275 and 280 nm by paper chromatography of an aliquot and by comparison with the authentic compounds and in the case of di and triphosphates by the determination of the labile phosphate content of these solutions according to the method of Fiske & Subbarow (4) The amount of the identified compounds was calculated from the absorbance of the concerning solutions at 265 nm against a blank obtained by mixing equal volumes of the fractions preceding and following the peak This had been justified since a linear gradient had been applied

Recovery experiments had been performed by addition of guanosine 5 monophosphate (GMP) and labelled adenosine triphosphate to the erythrocytes before haemolysing A mean recovery of 96 was found Definite calculations had been made with regard to this percentage

In every blood sample the haemoglobin content, the haematocrit value and the erythrocyte count were determined according to our current laboratory methods

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Table 1 *Concentration of purine nucleotides in the patients Re W and Ru W before and during oral administration of adenine*

	Haematocrit	MCHC	Nucleotides in µmoles per litre of free cell water[a]			
			AMP	ADP	ATP	GTP
Normal individuals Mean value ± 1 S D			37 9±2.03	224±30	1 830±110	103±10
Re W						
before adenine administration	38 0	33 0	32 2	118	1 500	114
during adenine administration	37 5	32 5	41 5	229	1 980	127
Ru W						
before adenine administration	33 5	33 0	35 7	111	1 580	120
during adenine administration	39 0	36 0	40 0	228	1 960	124

[a] The concentration of nucleotides per litre of free cell water is calculated according to Hjelm (7) by the formula

$$\text{conc /l of free cell water} = \frac{100 \quad \text{conc /l of packed cells}^{b}}{100\text{-MCHC}}$$

[b] The concentration per litre of packed cells can be calculated from the concentration per litre of whole blood and the haematocrit value

RESULTS

Table 1 shows the result obtained with erythrocytes of 12 children of varying ages without metabolic haematological neurological or mental disease and of two brothers Re W (9 years) and Ru W (2 years) suffering from the Lesch Nyhan syndrome

In both patients purine nucleotides had been determined before and after 32 weeks of oral administration of adenine During this period Re W obtained 200 mg (13 mg/kg) of adenine daily Ru W obtained during the first 7 weeks 160 mg daily (14 mg/kg) and the following 25 weeks 240 mg daily (22 mg/kg)

The following nucleotides had been identified in erythrocytes adenosine 5 monophosphate (AMP) adenosine-diphosphate (ADP) adenosine triphosphate (ATP) and guanosine triphosphate (GTP) Small amounts of inosine 5 monophosphate (IMP) and guanosine-diphosphate (GDP) (about 1 µmole/l blood) were sometimes found in the controls but were never found in the patients GMP inosine-diphosphate (IDP) and inosine triphosphate (ITP) were never found in detectable amounts (≥1 µmole/l blood)

The normal values found for AMP ADP ATP and GTP agree with the values reported

Because these nucleotides are water soluble the concentration per litre of free cell water is the best mode of expression giving the most information of the concentration of compounds involved in biochemical reactions (7)

Before adenine administration in both patients we found a decreased concentration of AMP ADP and ATP and a normal concentration of GTP

During oral administration of adenine normal values for AMP ADP and ATP were found GTP concentration was found normal or slightly increased

DISCUSSION

Several investigators searched for the cause of increased purine synthesis *de novo* in Lesch Nyhan syndrome Rosenbloom et al (16) discussed the following suppositions

1 The decreased resynthesis of GMP and IMP results in a higher availability of phosphoribosylpyrophosphate (PRPP) for the synthesis of phosphoribosylamine (Fig. 1)

2 In HGPRT ase deficient fibroblasts PRPP amidotransferase is activated by an increased concentration of hypoxanthine and guanine which are found to be inhibitors in normal cells (16)

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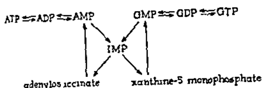


Fig 2 Interconversion of purine nucleotides

reactions in a cyclic interconversion of adenine and hypoxanthine derivatives and that a de ficiency of this enzyme results in a depletion of intracellular adenine nucleotides

Wyngaarden & Ashton (19) investigated the influence of the various purine nucleotides on the activity of PRPP amidotransferase obtained from pigeon liver ATP and ADP had the strongest inhibiting effect followed by GMP AMP IMP and GDP

Henderson (6) found in Ehrlich ascites tu mour cells a more potent inhibition by adenine than by guanine and showed that the purine bases have to be readily converted to ribonucleotides in order to exert their inhibiting effect

It is highly probable that in Lesch Nyhan pa tients the increased purine synthesis *de novo* is caused by the decreased concentration of ade nine nucleotides

A more detailed study has to be made on the feedback inhibition of the purine synthesis *de novo* in human tissue

If a real equilibrium exists between the three monophosphoribonucleotides AMP IMP and GMP (Fig 2) this balance must be disturbed in Lesch Nyhan syndrome causing a selective decrease of AMP resulting in a much larger decrease of ADP and ATP

SUMMARY

This paper presents the results of an investiga tion on the concentration of purine nucleotides in erythrocytes of two children with the Lesch Nyhan syndrome before and during oral ade nine administration The nucleotides AMP ADP ATP and GTP could be calculated Be fore administration the patients showed a de creased concentration of the three adenine nucleotides expressed as μmoles per litre of free cell water The concentration of GTP was found to be normal After oral adenine ad ministration adenine nucleotides were found to be normal and GTP showed a slight increase Some suppositions about the mechanism of increased purine synthesis *de novo* in patients with the Lesch Nyhan syndrome have been discussed

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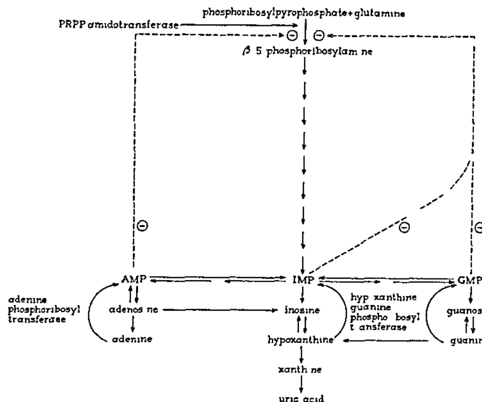


Fig 1 Purine synthesis de novo and purine metabolism

3 A decreased feedback inhibition of purine synthesis *de novo* caused by a decreased concentration of GMP and IMP Rosenbloom examined the purine nucleotides content of normal fibroblasts and those of patients with the Lesch Nyhan syndrome, and found no significant difference between both kinds of cells These fibroblasts were cultured in Eagle medium containing a large amount of glutamine which is a precursor in purine synthesis *de novo*

Ghadimi et al (5) concluded from the study of Seegmiller et al (17) that HGPRT ase deficient cells contain an increased amount of AMP However, this conclusion is based on an erroneous interpretation of the results of Seegmiller and co workers who only demonstrated that adeninephosphoribosyltransferase (APRT-ase) activity is higher in HGPRT ase deficient red cells than in normal cells

Manzke (12) and Labrune et al (10) determined the concentrations per litre of erythrocytes of AMP, ADP and ATP in patients with the Lesch-Nyhan syndrome Manzke found normal values but calls his methods in question himself Labrune and co workers found increased values for adenine nucleotides but the authors did not mention their methods of determination Furthermore these determinations are performed a few days after a blood transfusion

The present investigation demonstrates that erythrocytes from patients with the Lesch Nyhan syndrome contain a decreased amount of adenine nucleotides whereas the GTP content is normal After oral administration of adenine the adenine nucleotides were normalized and GTP showed a slight increase Previously (20) we showed that purine synthesis *de novo* in Lesch Nyhan patients was reduced during oral adenine administration as compared to the untreated patients The results focus our attention to the role of adenine nucleotides, since the concentration of GTP was found to be normal in the patients After administration of adenine the decrease of purine synthesis *de novo* could be caused by an increase of feed back inhibition by adenine nucleotides

The decrease of adenine nucleotides in our untreated patients can be explained according to Balis (2) and Sorensen (18) who postulated that HGPRT ase completes the sequence of

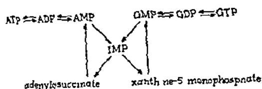


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ANAPHYLAXIS AND RED CELL SURVIVAL STUDIES IN A CHILD WITH INSULIN RESISTANT DIABETES MELLITUS

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A syndrome of lymphadenopathy hepatosplenomegaly Coombs positive hemolytic anemia and leukopenia in an insulin resistant juveline diabetic have previously been described by us (8 9) Immunological investigations showed that the child had high or normal immunoglobulin levels but that he was immunologically deficient as manifest by poor humoral antibody responses and defective cellular immunity (9) Clinical studies of the patient s serum revealed immune complexes of insulin anti insulin specificity and similar investigations in other diabetics have shown that such antigen antibody complexes are not uncommon in diabetic sera (4 14 15)

Immune complexes are known to lyse cells (11 20) and mediate anaphylactic reactions (12 13) It was therefore deemed important to study the patterns of erythrocyte sequestration and red cell survival in a diabetic known to have antigen antibody complexes in the circulation Also this was an opportunity to study the anaphylactic properties of the patient s serum The results showed that red cell survival is dependent on the type of insulin employed during therapy and that the anaphylactic properties of the antibody is of prognostic value in predicting the patient s response to corticosteroid therapy

MATERIALS AND METHODS

Red cell survival and sequestration studies were done with Cr labelled erythrocytes All survival curves were extended over 2 weeks to obtain accurate decay data and sequestration data was obtained by counting over the heart spleen and liver Passive cutaneous anaphylaxis (PCA) was done in guinea pigs (18) and rabbits (6) and vascular permeability was monitored with Evans blue (18) and an isotope tracer (7) Immune complexes were qualitatively identified by means of autoradiography (4 21) and were quantitatively measured according to a dialysis technique (14 15) Anti insulin antibodies were measured by the hydrodynamic flow method (10) IgG was prepared from the patient s serum by means of ion-exchange chromatography on DEAE cellulose using a Tris-phosphate continuous gradient elution scheme (5) Complement fixation was studied by means of passive immune lysis (19)

CASE REPORT

The patient is a 10 year old white male who was diagnosed as having diabetes mellitus in February 1963 In April 1963 he presented in ketoacidotic coma and was hospitalized when he was found to have hepatosplenomegaly and lymphadenopathy His anti insulin titer was 12 (normal <1) He was discharged on a regimen of 30 mg of prednisone and 170 units of pork insulin per day and the prednisone was discontinued in May 1964 In 1965 the patient was found to be hypergammaglobulinemic with normal IgA and IgM but elevated IgG (24 mg/ml normal 10 mg/ml) In 1966 the IgG was 13 5 mg/ml and he was receiving 75 units of pork insulin per day Lymphadenopathy and splenomegaly were recorded in February 1967 Serum IgG was elevated



Fig 1 Radioautograph of anti insulin activity before and after immune complex dissociation Whole serum on left and dissociated serum on right Patient s serum in the well and the trough contained I' insulin followed by rabbit anti human coprecipitating antibody

(14 mg/ml) but the insulin requirements had fallen to 54 units (pork) per day In August 1967 the patient was found to be pale and his hemogram revealed 6 g/100 ml of hemoglobin (Hgb) hematocrit (Hct) 18 normal platelets and a reticulocyte count of 10 6° The leukocyte count (WBC) was 1 850/mm³ with 20 neutrophils 72% lymphocytes 5 monocytes and 3 basophils The direct Coombs test was strongly positive A diagnosis of Coombs positive hemolytic anemia was established and an erythrocyte sequestration study was done before the institution of therapy Then a regimen of 40 mg prednisone and 63 units of pork insulin per day was begun Hemolysis ceased and the lymph nodes and spleen returned to normal The prednisone was stopped in January 1968 at which time the hemogram showed 13 2 g/100 ml Hgb 40% Hct and WBC 12 450 with 90 neutrophils and 10 lymphocytes The anti insulin titer was 9 the serum IgG was 7 2 mg/ml and the insulin requirements had fallen to 45 units (pork) per day In April 1968 the patient again presented with hepatosplenomegaly and lymphadenopathy and a hemogram showed 8 5 g/100 ml Hgb 25% Hct normal platelets and a WBC of 3 500 Prednisone was begun at 80 mg/day for 1 month It was then changed to 60 mg every other day for 2 weeks and was stabilized at 40 mg every other day In July 1968 the patient s hemogram lymph nodes liver and spleen were normal In 1969 a red cell survival study was performed while he was receiving either pork or de alaninated pork insulin He presently receives 71 units of pork insulin per day and 40 mg of prednisone every other day He has grown and done well in school and is presently in the 50 percentile for height and weight

It has been repeatedly observed that the patient s insulin requirements have increased when he has received prednisone The steroid has unquestionably controlled his hemolytic anemia and diminished the degree of hepatosplenomegaly and lymphadenopathy However his physicians have been regularly met with a 20-25 increase in the patient s insulin need during corticosteroid therapy Multiple marrow aspiration and lymph node biopsies have not supported a diagnosis of malignancy of the blood or blood forming organs Immunopathological immunohematological and immunochemical studies of this case have been published (9)

RESULTS

Demonstration of immune complexes

This has been extensively studied and reported in references (4 8, 9 14 15) The serum used for the anaphylaxis experiments had an anti insulin titer of 9 before antigen antibody dissociation and a titer of 27 after dissociation The post dissociation sample showed a longer and more dense antibody arc in the radioautograph than the pre dissociation sample as seen in Fig 1 Undiluted patient s serum (0 1 ml) injected intradermally into a blued guinea pig (3) caused a bluing reaction of 2+ intensity and 11 mm diameter Other diabetic sera used in the anaphylaxis assay were negative when diluted 1 10 in the blued guinea pig test Undiluted patient s serum provoked a



Fig 2 Passive cutan ous anaphylaxis From Table 1 7 8 and 9 are non diabetic serum (adult) non-diabetic serum (neonatal) and patient s IgG respectively Numbers 1 2 and 3 are the prednisone responsive insulin re sistant adult and child diabetic sera and 4 5 and 6 are 1 10 dilutions of 1 2 and 3 Note that the patient is negative

complement mediated passive immune lysis of sheep erythrocytes but the ptient s IgG ws negative in this test

Anaphylaxis experiments

PCA studies were done with an IgG prepara tion from the patient s serum Sera from three prednisone responsive insulin resistant patients were used as positive controls and two non diabetic sera were used as negative controls Guinea pigs were intradermally sensitized with 1 10 dilutions of the test and control sera and insulin Evans blue and 2 uCi of $I^{1\ 5}$ labelled guinea pig IgG were given intra venously 4 hours later The negative controls and the patient s IgG were negative but the positive controls showed qualitatively positive reactions as seen in Fig 2 These data are quantitatively expressed in Table 1 and they clearly show that the patient s antibody is negative in spite of its high titer of anti insulin activity The patient s IgG was again negative when studied in rabbits employing a 24 hour latent period (6)

Sequestration and survival of erythrocytes

Radiobiological experiments were done in 1967 to monitor the sites of red cell sequestration and in 1968 to determine the in vivo survival time for erythrocytes These experiments were done after the onset of hemolytic anemia

Table 1 *Passive cutaneous anaphylaxis as an index of prednisone responsiveness*

Samples	Anti insulin titer (10)	Passive cutaneous anaphylaxis		
		Intensity (18)	Millimeters (18)	Counts/Min (7)
Non-diabetic adult serum	0	0	0	52
Non-diabetic neonatal serum	0	0	0	41
Prednisone-responsive insulin resistant adult diabetic sera	8 5	2+	10	671
	13	3+	16	1 180
Prednisone responsive insulin resistant diabetic serum, child	15	3+	18	1 496
Patient s IgG	9[a]	0	0	67

Sera known to contain anti insulin antibodies of IgG specificity (4)

Table 2 *^{51}Cr RBC sequestration data*

Day of study	Body counts			Spleen/Liver Ratio
	Heart	Spleen	Liver	
1	1 940	3 874	1 964	2 5 1
2	1 919	3 452	1 497	2 3 1
5	1 367	3 103	1 163	2 7 1

(*a*) Red cell sequestration This investigation was done before institution of prednisone therapy, and results were compatible with a diagnosis of hypersplenism Counts over the heart, spleen, and liver for 5 days revealed a 2 7 1 0 ratio for spleen/liver as seen in Table 2

(*b*) Red cell survival Experiments were done after the diagnosis and control of the hemolytic process in order to study the relationship of the administered insulin to the patient's hemolysis Baseline determinations of red cell survival were done while the patient received pork insulin and prednisone A hypoallergenic insulin was then substituted for his usual insulin The modified product was pork insulin from which the C terminal amino acid alanine had been removed (1, 2) Erythrocyte survival studies were repeated after the patient had been receiving the de alaninated insulin for 1 month The data in Table 3 show that the erythrocytes experienced a longer survival while the patient was receiving his usual insulin than when he received the de alaninated insulin Body counts at this time revealed no splenic sequestration of red cells

Table 3 *Erythrocyte survival data*

Date	Insulin therapy	Label	RBC survival	
			Patient	Normal
Jan 1969	Pork	99 μCi ^{51}Cr	23 days	30±3 days
March	De alaninated pork		16 days	30±3 days

DISCUSSION

The PCA experiments produced positive reactions with several sera from prednisone responsive insulin resistant diabetics, but serum from the patient under study was consistently negative (Fig 2 and Table 1) Prednisone responsive diabetics are insulin resistant patients who require less insulin while undergoing steroid therapy (*16*) The patient under study required more insulin while receiving prednisone, and his anti insulin antibody was PCA negative These data are consistent with the Oakley hypothesis that sera from insulin-resistant diabetics who require less insulin during prednisone therapy demonstrate positive PCA reactions, and insulin resistant diabetics requiring more insulin during prednisone therapy give negative PCA reactions (17) The test is a useful prognostic adjunct in determining whether or not an insulin resistant diabetic is going to respond to prednisone

The hemolytic process was controlled with oral prednisone therapy, but the underlying pathophysiology was only suppressed and not corrected Adequate suppression was demonstrated in the red cell sequestration studies where splenic trapping was demonstrated before but not after corticosteroid therapy (Table 2) The reappearance of lymphadenopathy and hemolysis with efforts to discontinue prednisone are corroborative clinical evidence that the basic disease was dampened but not eliminated by steroids

The red cell survival experiments done whi[le] the patient was receiving pork and de alani[n]ated insulin was used The substitution of de alaninated insulin for the patient's usual in sulin created a situation in which immuno globulins with specificity for the C termina[l] amino acid of pork insulin could no longe[r] combine with their antigen in vivo The ne[t] effect of this change was an elevation in th[e] anti insulin titer This conclusion is strengthened by the fact that we have now documented similarly elevated anti insulin titers following insulin changes while cycling another diabetic through beef pork and fish insulins

(14 15) Since the patient s hemolytic anemia is causally related to his anti insulin antibodies (9) it is not surprising that his red cell survival decreased when the insulin type was changed (Table 3) This represents the first demonstration that erythrocyte survival in diabetes is dependent on the type of insulin employed during therapy

SUMMARY

A child with the syndrome of diabetes mellitus insulin resistance Coombs positive autoimmune hemolytic anemia lymphadenopathy and hepatosplenomegaly was described The patient s IgG anti insulin antibody was shown to be negative for passive cutaneous anaphylaxis (PCA) In as much as the pati nt was refractory to prednisone therapy and presented a negative PCA test he was consistent with the Oakley hypothesis that insulin resistant prednisone insensitive patients will be negative for PCA The half life of the patient s red blood cells was shown to vary as a function of the type of insulin used *in therapy* The child was shown to have immune-complexes in his serum of insulin anti insulin specificity and it was suggested that these complexes are partially responsible for the microangiopathy that is so often observed in diabetes mellitus

ACKNOWLEDGEMENTS

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The red cell survival experiments done while the patient was receiving pork and de alaninated insulin was used The substitution of de alaninated insulin for the patient s usual insulin created a situation in which immunoglobulins with specificity for the C terminal amino acid of pork insulin could no longer combine with their antigen in vivo The net effect of this change was an elevation in the anti insulin titer This conclusion is strengthened by the fact that we have now documented similarly elevated anti insulin titers following insulin changes while cycling another diabetic through beef pork and fish insulins

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AN EPIDEMIOLOGICAL STUDY OF CHILD HEALTH AND NUTRITION IN A NORTHERN SWEDISH COUNTY

III *Medical and anthropometrical examinations*

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In recent years fairly extensive epidemiological studies of the health status of children in Sweden have been published by Mellander et al (15) Mellbin (16) Sterky (22) and Karlberg et al (11) These studies have mainly been concerned with special groups in the population and with infants and children up to three years of age

The aim of this investigation was to study the health and nutritional status of three age groups of children in different parts of a northern Swedish county at the same time that an investigation of food intake was conducted (18) and to relate the findings to food habits and socio-economic factors

MATERIAL

All of the 1 401 children investigated were selected from three areas of Vasterbotten county which differ from one another geographically and in part socio-economically namely one urban area and two rural areas (Fig 1) All children were selected from the population register for Vasterbotten county A detailed description of the selection procedure is given by Samuelson 1971 (18) Three age groups were studied. 4- 8- and 13 year-olds with exact decimal median ages of 4 378 years 8 296 years and 13 317 years In all three areas 8 and 13 year-olds were studied, while in the city of Umeå also 4-year-olds were includ d The composition of the material and its age and sex distribution is shown in Table 1 the reasons for the omission of certain children in Table 2

All urban children were from the city of Umeå near the coast of the Gulf of Bothnia Every second child in the age groups, 4 8 and 13 years was selected from the population register for the parts of the city of Umeå proper

In the inland area which is rural all children in the 8 and 13 year-old groups were included For practical reasons the study included only school children in this area

In the third area the mountain foreland as in the inland area all 8 and 13 year-olds were included

Details about the geography and population are given by Samu lson 1971 (18)

METHODS

The medical examination

All children underwent a medical examination All the examinations were performed during the autumn of 1967

The same paediatrician (the author) examined all the children in the study and personally performed all the measurements and assessments The preschool children were examined in a child health centre in the city of Umeå All the school children were examined at school under comparable conditions in the three areas The same equipment was used throughout the study The medical examination of a child took an average of 10–15 min

1 The physical examination

The physical examination was performed according to a previously d termined scheme The children s general condition was not graded The mouth lips and throat were examined and angular lesions were recorded Conjunctivitis or other changes in the conjunctivae were noted. Upper respiratory infections such as pharyngitis and signs of the common cold were recorded The teeth and gums were not included in this examination but a special odontological examination was performed by a dentist imm diately before or after the medical examination This part of the study is described by Samuelson et al (19)

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Key words Diabetes mellitus insulin resistance passive cutaneous anaphylaxis erythrocyte survival immune complexes

Malmo General Hospital according to an immunochemical method described by Laurell (13)

4 Anthropometrical measurements

All the measurements on a given child were performed on the sam occasion. Internationally accepted techniques were used (3 7 10 24)

Weight

All children were weighed on the same platform balance (Stathmos 304 AB Lindell Jonkoping Swe den) This balance is robust and easy to transport It was protected from mechanical damage in tran sport by a 4 cm thick sheetrubber carpet Before use the zero setting of the balance was checked with 10 and 20 kg w ights and this check was repeated 4-5 times each day Control weighings of the weights at the National Commission of Standards showed that they differed by no more than +3 g from 10 and 20 kg respectively

During the investigation the balance was checked each month at the workshop of Umea University Hospital

The children were weighed naked or wearing only light underwear They stood on the centre of the plat form. All weighings were done by the same person who recorded the weights to the nearest 0 1 kg

Height

For measuring the 4 year olds a scale fixed to a wall was used Th child stood barefoot on the floor with the feet parallel and the heels buttocks and back of the head touching the wall The arms were at the sides A wooden headpiece was lowered until it touched the top of the head The measurement was recorded to within 0 5 cm

The school children were measured using a vertical metal measuring rod attached to the platform balance used for weighing The children stood on the plat form with the feet parallel and with the heels at the end of the platform in the same position for each child The buttocks shoulders and back of the head were in contact with the rod and the arms were at th sides The top of the metal rod bent 90 was lowered until it touched the top of the head The measurements were recorded to within 0 5 cm The measuring scale was regularly checked by comparing th result of measuring a child with this scale with that obtained using a scale fixed to the wall All measurements were carried out by the same person

Subcutaneous fat

For determination of skinfold thickness a skinfold caliper (model Harpenden British Indicators Limited) was used. The determinations were performed by the same person using the same caliper for all children The Harpend n caliper exerted a constant pressure of 10 g/mm By interpolation between the markings on the dial thickness was measured to the n arest 0 1 mm

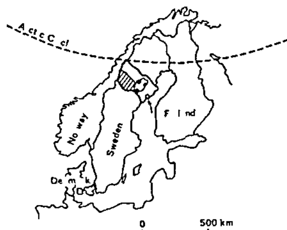


Fig 1 A map of Scandinavia showing the county of Vasterbotten and the three areas studied The arrow indicates the city of Umeå the stippled area the in land area the shaded area the mountain foreland

The triceps skinfold was measured in the middle part of th left arm at a line between the tip of the acromion process of the scapula and the olecranon process of the ulna The longitudinal skinfold and the subcutaneous tissue was picked up between the thumb and forefinger of the left hand drawn away from the und rlying muscle and measured Three measurements were taken and the mean value of these was recorded

The subscapular skinfold was measured just below and lateral to the angl of the left scapula A skin fold was picked up parallel to the natural cleavage line of the skin. Three measurements were taken and the mean value of th se was recorded

For both triceps and subscapular skinfolds the error of a single determination was calculated from 100 duplicate determinations in each age group[1] and was found to be ±0 1 mm for all age groups

Data on ESR are lacking for 3 % of the 8 year-olds and 2% of the 13 year olds Serum albumin levels are lacking for 17% of the 4 year-olds 23% of the 8 year-olds and 20 of the 13 year olds Values for these children are missing because of technical dif ficulties, for example hemolysis in the s rum or the destruction of micro-capillary tubes in transport In the 4 year-old group data on skinfold thickness are lacking for 24% of the children The measurements on these 47 children were not included because initial difficulty in repeatedly measuring the skinfold led to uncertain values

Statistical methods

The methods used are described elsewhere (18)

[1] According to $\sigma = \pm\sqrt{\Sigma d^2/2n}$ where d is the difference between duplicate determinations and n is the number of the differences

Table 1 *Age, sex and geographical distribution of the 1401 children studied*

Age group Median age	4-year olds 4 years 5 months			8 year olds 8 years 4 months			13 year olds 13 years 4 months		
	Boys	Girls	Total	Boys	Girls	Total	Boys	Girls	Total
City of Umeå	99	99	198	100	100	200	100	100	200
Inland area	—	—	—	98	90	188	91	111	202
Mountain foreland	—	—	—	99	96	195	114	104	218
Total	99	99	198	297	286	583	305	315	620

Pathological findings in the superficial lymph nodes in the neck and in the axillae were recorded The thyroid gland was examined for enlargement

Dermatoses and allergic and infectious manifestations in the skin were noted Unusual properties of the hair such as thinness and dyspigmentation were looked for The nails were examined for brittleness

The blood pressure and the heart rate were not registered but pathological auscultation findings in the heart and lungs were recorded

The abdomen was palpated for tumors or possible enlargement of the liver or spleen

The muscle mass was not recorded but signs of muscular dystrophy or wasting were looked for

The genital organs of boys were examined for undescended testes

Abnormal findings in the skeletal system such as deformities Harrison s sulcus of the chest and other aberrations were looked for The general gait was observed in all children

2 *Microsedimentation rate (ESR)*

ESR was determined according to Strom (23) Within two hours of blood sampling the mixture of blood and anticoagulant was drawn into micropipettes for the determination The room temperature varied between 18 and 22 C

3 *Albumin concentration*

Albumin was determined on serum from capillary blood samples The samples were drawn from finger pricks into heparinized capillary tubes and were centrifuged in an International Micro-Capillary Centrifuge The centrifugation time was 4-5 min and the speed 11 000 r p m The albumin determinations were performed at the Department of Clinical Chemistry

Table 2 *Distribution of omissions in the different areas*

1 = City of Umeå 2 = Inland area 3 = Mountain foreland

	1	2	3	Total	Per cent
Invited	635	411	442	1488	100 0
Moved	29[a]	12	18	59	4 0
Remained to examine	606	399	424	1429	96 0
Non Caucasian (age uncertain)	1	—	—	1	0 1
Mental retardation	5	3	6	14	1 0
Blindness (cerebromacular degeneration)	—	—	1	1	0 1
Diabetes mellitus	—	3	1	4	0 3
Chondrodystrophy	—	1	—	1	0 1
Remained to examine	600	392	416	1408	94 6
Refused	—	—	2	2	0 1
Excluded because food consumption at recall was incomplete due to					
Acute gastroenteritis	2	—	1	3	0 2
Upper respiratory infection	—	2	—	2	0 1
Final material taking part in the investigation	598	390	413	1401	94 2

[a] Of these fifteen were 4 year olds

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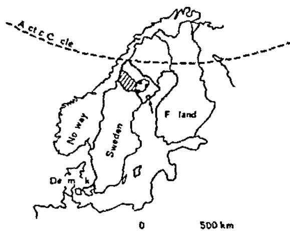


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According to $\sigma = +\sqrt{\Sigma d^2/2n}$ where d is the difference between duplicate determinations and n is the number of the differences

Table 3 *Summary of clinical findings in the examination*

	4 year olds		8 year olds		13 year olds	
Diagnosis	No		No		No	
Upper respiratory infection	22	11 1	28	4 8	38	6 1
Bronchitis	2	1 0	5	0 9	1	0 2
Acute conjunctivitis	1	0 5	2	0 3	0	—
Angular lesions	0	—	5	0 9	2	0 3
Enlargement of the thyroid gland	0	—	0	—	9	1 5
Eczema	12	6 1	3	0 5	13	2 1
Impetigo	0	—	3	0 5	5	0 8
Valvular aortic stenosis	0	—	0	—	1	0 2
Ventricular septal defect	0	—	0	—	1	0 2
Inguinal hernia	0	—	2	0 3	0	—
Hydrocele testis	1	1 0	0	—	0	—
Undescended testis	2	2 0	4	1 3	2	0 7
Hypospadias	0	—	1	0 2	1	0 2
Minor cerebral palsy	0	—	1	0 2	2	0 3

RESULTS

I *Medical examination*

The results of the medical examination are shown in Table 3

4 year old group The general nutritional status was good for all children in the group Upper respiratory infections (rhino naso-pharyngitis and/or enlarged and tender superficial lymph nodes in the neck) were noted in 11 1% at the time of examination Mild eczema, mainly in the bend of the arm and on the cheeks, was present in 6 1% At the time of the examination, no child had severe or weeping eczema

Physiological heart murmurs were registered but have not been included in the summary of the findings No hepatic or splenic enlargement was found

8 year-old group The general nutritional status was good for all children Upper respiratory infections were noted in 4 8% of the children at the time of examination There were dry eczematous skin changes in 0 5% of the children

No hepatic or splenic enlargement was found

One child had partial hemiparesis due to birth injury, but the damage was not so severe as to constitute a handicap

Comparison between the different areas revealed no differences in general health status The frequency of upper respiratory infections and eczema was about the same in all areas

13-year-old group The general nutritional status was good Upper respiratory infections were present in 6 1% of the children at the time of examination Thyroid enlargement was found in 1 5% of the 13-year olds, all girls Skin changes of an eczematous type were present in 2 1% of the children Auscultation of the heart revealed that two children had systolic murmurs of a pathological type Previous investigation at the Department of Paediatrics, Umea, had established that one of these children had subvalvular aortic stenosis and the other had a ventricular septal defect Both children were free of subjective symptoms

No hepatic or splenic enlargement was found Two children had inguinal hernias

Two children had non handicapping residual neurological damage resembling mild cerebral palsy

One child was found to have moderate icterus Later investigation showed that this was familial, non hemolytic icterus (Gilbert s disease)

Comparison between the different areas revealed no differences in the general health status and no differences in the frequency of upper

Table 4 *Mean values and standard deviations (S D) of ESR in mm*

	4-year olds			8 year-olds			13-year-olds		
	No	Mean	S D	No	Mean	S D	No	Mean	S D
Boys	97	18	12	289	13	10	297	13	9
Girls	95	21	12	275	14	9	311	15	9
Total	192	20	12	564	14	10	608	14	9

respiratory infections The frequency of eczema was the same in all areas Thyroid enlargement was found only in 13 year-old girls in the inland area (5 cases) and in the mountain foreland (4 cases)

II. *Microsedimentation rate (ESR) and serum albumin*

4 year old group There was no significant sex difference in the mean value of the ESR (Table 4) Of the 4-year-olds 38% had an ESR above 20 mm The mean value for the ESR in this a e group was significantly higher than the corresponding mean values in the 8 and 13 year old groups ($p<0.01$)

The means and standard deviations for serum albumin levels are given in Table 5

8 year-old group There was no significant sex difference in the mean value of the ESR (Table 4)

On comparison between the different areas it appeared that 16% of the children in the city of Umeå 12% in the inland area and 21% in the mountain foreland had ESR values above 20 mm The children in the mountain foreland had a higher mean ESR value than the children in the inland area ($p<0.05$)

There were no significant differences in the serum albumin levels either between the sexes or between the different areas (Table 5)

13 year-old group As a group the girls had a somewhat higher mean ESR than the boys ($p<0.01$)

Comparison between the different areas revealed no significant differences in the mean ESR values ESR values above 20 mm were found in 18% of the children in the city of Umeå 19% in the inland area and 21% in the mountain foreland

The mean value for serum albumin which is shown for the group as a whole in Table 5 did not differ between the sexes or between the different areas

III *Anthropometrical measurements*

Table 6 *a–d* shows the means and standard deviations and Fig 2 *a–d* the percentiles for weight height and triceps and subscapular skinfold thickness

4 year-old group There were no significant sex differences in weight and height On the other hand the mean values for triceps and subscapular skinfold thickness were higher in girls than in boys ($p<0.01$)

Table 5 *Mean values and standard deviations (S D) of albumin in serum of 4 8 and 13 year-old children*

The values are given in g/100 ml

	4-year-olds			8 year-olds			13-year-olds		
	No	Mean	S D	No	Mean	S D	No	Mean	S D
Boys	83	4.34	0.40	216	4.42	0.39	238	4.56	0.38
Girls	81	4.31	0.38	231	4.52	0.42	256	4.51	0.34
Total	164	4.33	0.39	477	4.47	0.41	494	4.54	0.36

Table 3 *Summary of clinical findings in the examination*

	4 year olds		8 year olds		13 year olds	
Diagnosis	No		No		No	
Upper respiratory infection	22	11 1	28	4 8	38	6 1
Bronchitis	2	1 0	5	0 9	1	0 2
Acute conjunctivitis	1	0 5	2	0 3	0	—
Angular lesions	0	—	5	0 9	2	0 3
Enlargement of the thyroid gland	0	—	0	—	9	1 5
Eczema	12	6 1	3	0 5	13	2 1
Impetigo	0	—	3	0 5	5	0 8
Valvular aortic stenosis	0	—	0	—	1	0 2
Ventricular septal defect	0	—	0	—	1	0 2
Inguinal hernia	0	—	2	0 3	0	—
Hydrocele testis	1	1 0	0	—	0	—
Undescended testis	2	2 0	4	1 3	2	0 7
Hypospadias	0	—	1	0 2	1	0 2
Minor cerebral palsy	0	—	1	0 2	2	0 3

RESULTS

I *Medical examination*

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Physiological heart murmurs were registered but have not been included in the summary of the findings No hepatic or splenic enlargement was found

8 year old group The general nutritional status was good for all children Upper respiratory infections were noted in 4 8% of the children at the time of examination There were dry eczematous skin changes in 0 5% of the children

No hepatic or splenic enlargement was found

One child had partial hemiparesis due to birth injury, but the damage was not so severe as to constitute a handicap

Comparison between the different areas revealed no differences in general health status The frequency of upper respiratory infections and eczema was about the same in all areas

13-year-old group The general nutritional status was good Upper respiratory infections were present in 6 1% of the children at the time of examination Thyroid enlargement was found in 1 5% of the 13 year olds, all girls Skin changes of an eczematous type were present in 2 1% of the children Auscultation of the heart revealed that two children had systolic murmurs of a pathological type Previous investigation at the Department of Paediatrics, Umeå had established that one of these children had subvalvular aortic stenosis and the other had a ventricular septal defect Both children were free of subjective symptoms

No hepatic or splenic enlargement was found Two children had inguinal hernias

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One child was found to have moderate icterus Later investigation showed that this was familial non hemolytic icterus (Gilbert s disease)

Comparison between the different areas revealed no differences in the general health status and no differences in the frequency of upper

Table 4. *Mean values and standard deviations (S.D.) of ESR in mm*

	4-year-olds			8 year-olds			13 year-olds		
	No	Mean	S.D.	No	Mean	S.D.	No	Mean	S.D.
Boys	97	18	12	289	13	10	297	13	9
Girls	95	21	12	275	14	9	311	15	9
Total	192	20	12	564	14	10	608	14	9

respiratory infections. The frequency of eczema was the same in all areas. Thyroid enlargement was found only in 13 year old girls in the inland area (5 cases) and in the mountain foreland (4 cases).

II. *Microsedimentation rate (ESR) and serum albumin*

4-year-old group. There was no significant sex difference in the mean value of the ESR (Table 4). Of the 4-year-olds 38 % had an ESR above 20 mm. The mean value for the ESR in this age group was significantly higher than the corresponding mean values in the 8- and 13 year old groups ($p < 0.01$).

The means and standard deviations for serum albumin levels are given in Table 5.

8 year-old group. There was no significant sex difference in the mean value of the ESR (Table 4).

On comparison between the different areas it appeared that 16 % of the children in the city of Umeå, 12 % in the inland area and 21 % in the mountain foreland had ESR values above 20 mm. The children in the mountain foreland had a higher mean ESR value than the children in the inland area ($p < 0.05$).

There were no significant differences in the serum albumin levels, either between the sexes or between the different areas (Table 5).

13 year-old group. As a group, the girls had a somewhat higher mean ESR than the boys ($p < 0.01$).

Comparison between the different areas revealed no significant differences in the mean ESR values. ESR values above 20 mm were found in 18 % of the children in the city of Umeå, 19 % in the inland area and 21 % in the mountain foreland.

The mean value for serum albumin, which is shown for the group as a whole in Table 5, did not differ between the sexes or between the different areas.

III. *Anthropometrical measurements*

Table 6 *a–d* shows the means and standard deviations and Fig. 2 *a–d* the percentiles for weight, height and triceps and subscapular skinfold thickness.

4 year-old group. There were no significant sex differences in weight and height. On the other hand, the mean values for triceps and subscapular skinfold thickness were higher in girls than in boys ($p < 0.01$).

Table 5. *Mean values and standard deviations (S.D.) of albumin in serum of 4-, 8- and 13 year old children*

The values are given in g/100 ml.

	4-year-olds			8 year-olds			13 year olds		
	No	Mean	S.D.	No	Mean	S.D.	No	Mean	S.D.
Boys	83	4.34	0.40	216	4.42	0.39	238	4.56	0.38
Girls	81	4.31	0.38	231	4.52	0.42	256	4.51	0.34
Total	164	4.33	0.39	477	4.47	0.41	494	4.54	0.36

Table 6*a* *Total material Weight height and skinfold thickness (Mean values and S D) in 4 8, and 13 year old children*

	No	Weight kg Mean	S D	Height cm Mean	S D	No	Triceps skinfold mm Mean	S D	Subscapular skinfold mm Mean	S D
4 year olds										
Boys	99	18 1	2 4	107 0	4 7	88	8 6 **	1 9	5 0 **	1 2
Girls	99	17 9	2 4	106 5	4 2	63	9 4	1 8	5 5	1 3
Total	198	18 0	2 4	106 8	4 5	151	8 9	1 9	5 2	1 2
8 year olds										
Boys	297	26 9	4 3	129 6	5 5	297	8 2 ***	2 6	5 1 ***	2 1
Girls	286	26 8	4 6	129 3	5 6	286	10 1	3 3	6 5	3 5
Total	583	26 8	4 5	129 5	5 5	583	9 1	3 1	5 8	2 9
13 year olds										
Boys	305	45 3 ***	8 9	157 0	8 2	305	8 9 ***	3 4	6 4 **	3 3
Girls	315	47 8	8 0	158 0	6 7	315	12 1	4 4	8 6	4 0
Total	620	46 6	8 5	157 5	7 5	620	10 5	4 4	7 5	3 8

** $p<0\ 01$ *** $p<0\ 001$

8 year-old group There were no significant sex differences in weight and height (Table 6 *a*) However the girls had significantly higher mean values for skinfold thickness than the boys ($p<0\ 001$)

Comparison between the different areas revealed the following

Weight The girls in the city of Umea weighed more ($p<0\ 05$) than the girls in the mountain foreland (Table 6 *e*)

Height The girls in the city of Umea were somewhat taller than the girls in the inland area or the mountain foreland ($p<0\ 05$) The 8 year olds were significantly taller ($p<0\ 01$) in

Table 6*b* *City of Umea Weight, height and skinfold thickness (Mean values and S D) in 8 and 13 year old children*

	No	Weight kg Mean	S D	Height cm Mean	S D	Triceps skinfold mm Mean	S D	Subscapular skinfold mm Mean	S D
8 year olds									
Boys	100	26 9	4 1	130 2	5 3	8 6 ***	2 5	5 3 ***	1 9
Girls	100	27 4	4 6	130 4	5 6	11 0	3 4	7 2	4 0
Total	200	27 2	4 4	130 3	5 5	9 8	3 2	6 2	3 3
13 year olds									
Boys	100	46 0	9 4	157 6	8 2	9 4 ***	3 9	6 9 **	3 8
Girls	100	47 3	7 9	158 9	6 3	12 0	4 2	8 6	4 3
Total	200	46 7	8 7	158 2	7 4	10 7	4 2	7 8	4 1

** $p<0\ 01$ *** $p<0\ 001$

Table 6c *Inland area Weight height and skinfold thickness (Mean values and S D) in 8 and 13 year-old children*

	No	Weight kg		Height cm		Triceps skinfold mm		Subscapular skinfold mm	
		Mean	S D	Mean	S D	Mean	S D	Mean	S D
8 year-olds									
Boys	98	27 0	4 1	129 8	5 7	8 1 **	2 6	5 0 **	1 8
Girls	90	26 7	5 5	128 8	5 7	9 7	3 5	6 5	4 0
Total	188	26 9	4 8	129 3	5 7	8 8	3 2	5 7	3 2
13-year-olds									
Boys	91	46 0	9 0	157 6	8 5	8 9 **	4 2	6 5 **	3 6
Girls	111	48 5	8 4	158 2	6 7	12 5	4 9	9 0	4 0
Total	[illegible]02	47 3	8 8	158 0	7 5	10 9	4 9	7 9	4 0

$p<0.05$ $p<0.01$ *** $p<0.001$

the city of Umeå than in the mountain foreland (Table 6 *e*)

Triceps skinfold Boys in the city of Umea had a significantly higher mean value for triceps skinfold than the boys in the mountain foreland ($p<0.05$) Girls in the city of Umea had a higher mean value for triceps skinfold than girls in the inland area ($p<0.01$) or the mountain foreland ($p<0.001$) (Table 6 *e*)

Subscapular skinfold This skinfold measurement was greater for girls in the city of Umea than for girls in the mountain foreland ($p<0.01$) The same significant difference was also found for the group as a whole (Table 6 *e*)

13 year-old group The girls had higher mean values for body weight and skinfold thickness than the boys (Table 6 *a*) There was no significant sex difference in height

Comparison between the different areas revealed the following

Weight There were no significant differences between the children in the different areas with respect to body weight

Height The mean height for the entire 13 year-old group in the city of Umea was higher ($p<0.05$) than that for the group in the mountain foreland (Table 6 *e*)

Triceps skinfold No significant differences

Table 6d *Mountain foreland Weight height and skinfold thickness (Mean values and S D) in 8 and 13 year old children*

	No	Weight kg		Height cm		Triceps skinfold mm		Subscapular skinfold mm	
		Mean	S D	Mean	S D	Mean	S D	Mean	S D
8 year-olds									
Boys	99	26 8	4 7	129 0	5 4	7 8 *	2.5	5 1	2.5
Girls	96	[illegible]6 1	3 7	1[illegible]8 5	5 3	9 4	2 8	5 8	1 8
Total	195	[illegible]6 5	4.2	1[illegible]8 8	5.3	8 6	2 8	5 4	2 2
13 year-olds									
Boys	114	44 2	8.2	156 0	7 8	8 4	[illegible]4	5.9	2.2
Girls	104	47 6	7 7	157 0	7 1	11 9	4 0	8.2	3 9
Total	218	45 8	8 2	156 5	7 5	10 1	4 1	7 0	3.3

$p<0.05$ $p<0.01$ $p<0.001$

Table 6*a* *Total material Weight, height and skinfold thickness (Mean values and S D) in 4 8, and 13 year old children*

	No	Weight kg Mean	S D	Height cm Mean	S D	No	Triceps skinfold mm Mean	S D	Subscapular skinfold mm Mean	S D
4 year olds										
Boys	99	18.1	2.4	107.0	4.7	88	8.6**	1.9	5.0**	1.2
Girls	99	17.9	2.4	106.5	4.2	63	9.4	1.8	5.5	1.3
Total	198	18.0	2.4	106.8	4.5	151	8.9	1.9	5.2	1.2
8 year olds										
Boys	297	26.9	4.3	129.6	5.5	297	8.2***	2.6	5.1***	2.1
Girls	286	26.8	4.6	129.3	5.6	286	10.1	3.3	6.5	3.5
Total	583	26.8	4.5	129.5	5.5	583	9.1	3.1	5.8	2.9
13 year olds										
Boys	305	45.3***	8.9	157.0	8.2	305	8.9***	3.4	6.4**	3.3
Girls	315	47.8	8.0	158.0	6.7	315	12.1	4.4	8.6	4.0
Total	620	46.6	8.5	157.5	7.5	620	10.5	4.4	7.5	3.8

** $p<0.01$ *** $p<0.001$

8 year-old group There were no significant sex differences in weight and height (Table 6 *a*) However the girls had significantly higher mean values for skinfold thickness than the boys ($p<0.001$)

Comparison between the different areas revealed the following

Weight The girls in the city of Umeå weighed more ($p<0.05$) than the girls in the mountain foreland (Table 6 *e*)

Height The girls in the city of Umeå were somewhat taller than the girls in the inland area or the mountain foreland ($p<0.05$) The 8 year olds were significantly taller ($p<0.01$) in

Table 6*b* *City of Umeå Weight height and skinfold thickness (Mean values and S D) in 8 and 13 year old children*

	No	Weight kg Mean	S D	Height cm Mean	S D	Triceps skinfold mm Mean	S D	Subscapular skinfold mm Mean	S D
8 year-olds									
Boys	100	26.9	4.1	130.2	5.3	8.6***	2.5	5.3***	1.9
Girls	100	27.4	4.6	130.4	5.6	11.0	3.4	7.2	4.0
Total	200	27.2	4.4	130.3	5.5	9.8	3.2	6.2	3.3
13 year olds									
Boys	100	46.0	9.4	157.6	8.2	9.4***	3.9	6.9**	3.8
Girls	100	47.3	7.9	158.9	6.3	12.0	4.2	8.6	4.3
Total	200	46.7	8.7	158.2	7.4	10.7	4.2	7.8	4.1

** $p<0.01$, *** $p<0.001$

age groups the serum albumin levels suggest that there was adequate protein intake which was confirmed in the food consumption survey (18) In this investigation serum albumin levels were determined as a complement to the clinical and anthropometrical investigations However it is to be observed that serum albumin de creases late in protein deficiency states There are still no biochemical methods for detecting early and slight degrees of malnutrition which are suitable for use in field studies on a large scale (10)

The results of the anthropometrical investiga tion height weight and skinfold thickness con firm that the nutritional state of the investigated children in all three age groups was good It is interesting that the girls in the 13 year-old group had a significantly higher mean body weight than the boys The difference was found in all three areas but it was significant only in the two rural areas (Table 6 *a–d*) It probably reflects the fact that girls engage in less physical activity than boys Although the girls in all areas consumed less than the boys (18) they probably did not expend their total energy in take In the younger age groups there were no significant sex differences in weight Another observation is that the girls in all the age groups had significantly greater mean skinfold thick ness than the boys This is consistent with the findings in other investigations (6 10 11 12 24)

As expected weight and skinfold thickness showed significant deviation from normal distri bution due to positive skewness (Figs 2 *a c* and *d*) However tests of significance of the differences between both the means and the medians gave the same result The coefficients of correlation (*r*) between triceps and subscap ular skinfold thickness were 0 56 for the chil dren in the 4 year-old group and 0 79 and 0 83 for the 8 and 13 year-old groups respectively The coefficients of correlation (*r*) between tri ceps and subscapular skinfold respectively and weight were for the children in the 4-year-old group 0 37 and 0 32 respectively In the 8 and 13 year-old groups on the other hand the coef

Table 6*e* *t Test on the difference of the means of weight height triceps and subscapular skin folds in the different age groups and geographic areas*

1 – City of Umeå, 2 – Inland area 3 – Mountain foreland

	Weight	Height	Triceps skinfold	Sub-scapular skinfold
8 year-olds				
Boys				
1 vs 2				
1 vs 3			*	
2 vs 3				
Girls				
1 vs 2			*	
1 vs 3	*	*	***	**
2 vs 3				
Total				
1 vs 2				
1 vs 3		**	***	**
2 vs 3				
13 year-olds				
Boys				
1 vs 2				
1 vs 3				*
2 vs 3				
Girls				
1 vs 2				
1 vs 3				
2 vs 3				
Total				
1 vs 2				
1 vs 3				
2 vs 3				*

* $p<0\ 05$ ** $p<0\ 01$ *** $p<0\ 001$ 1>2 1>3 2>3

ficients were 0 66 0 68 and 0 61, 0 66 respec tively The results for the school children are in fairly good agreement with those of Corbin on American elementary school children (4) The lower correlation in the 4 year-old group may be real or may be due to technical diffi culties in measuring skinfolds in preschool chil- dren

That the children in the city of Umea were somewhat taller than the children in the moun tain foreland may be due to nutritional differ ences between areas that differ socio-economic- ally (18) There may have been geographical differences in nutritional intake in Vasterbotten early in the lives of the investigated children and/or in their mothers It cannot be estab-

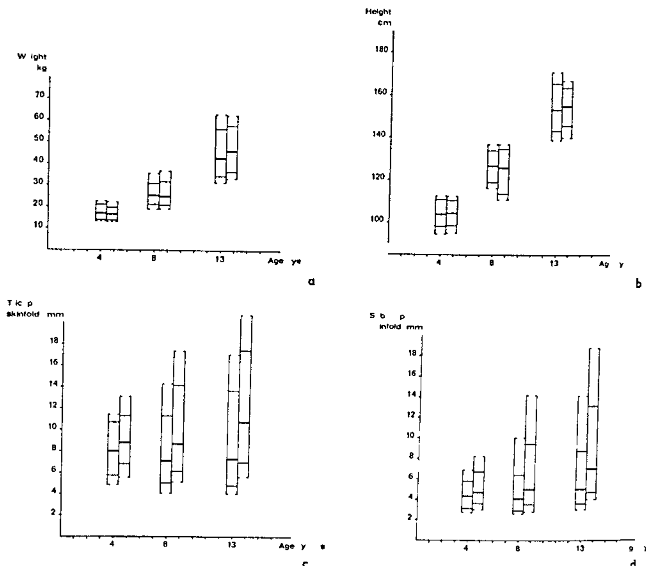


Fig 2 Percentile distribution of (*a*) weight (*b*) height (*c*) triceps skinfold and (*d*) subscapular skinfold in 4 8 and 13 year olds The 50th percentile is indicated by a heavy line the 90th and 10th percentiles are indicated by the solid lines above and below and the 97th and 3rd percentiles by the dotted lines at the extremes Values for boys at the left values for girls at the right

were found between children in the different areas

Subscapular skinfold Boys in the city of Umea had a higher mean value for the subscapular skinfold than the boys in the mountain foreland ($p<0\ 05$) The children in the inland area, in turn had a somewhat higher mean value for subscapular skinfold ($p<0\ 05$) than the children in the mountain foreland (Table 6 *e*)

DISCUSSION

The aim of the medical examination was to determine the physical health status of the children who took part in the food consumption survey (18), and to relate the findings to food habits and socio economic conditions (21) As expected no case of clinical undernutrition was found a wide variety of foods are available in the regions studied and the economic standard of the population is fairly high It should be pointed out however that the methods used for evaluating health and nutritional status are rough A clinical examination is subjective and depends on the experience and thoroughness of the investigator However since the same investigator examined all the children comparisons between the areas should be valid In all

economic conditions for growth and physical development has been demonstrated in a number of studies (e g 5 6 8, 11)

In the mountain foreland the mean values for skinfold thickness were lower than those in the city of Umeå and to some extent even than those in the inland area Since the food consumption survey (18) showed that children in both the mountain foreland and the inland area had higher energy intakes than the children in the city of Umeå the difference in skinfold measurement can hardly be due to a difference in nutritional intake One is inclined to assume that this difference is mainly due to less physical activity on the part of children in Umeå Further a number of factors suggest that children in the city of Umeå engage in less physical activity in their daily lives They have shorter distances to school and it is likely that their spare time activities include less physical activity whereas children in the mountain foreland probably spend more time out of doors and engage in more fresh air sports than city children The role of inactivity in the etiology of obesity especially in the growing child has been pointed out by Mayer (14) Parizkova (17) and others

Compared with earlier Swedish findings by Broman Dahlberg & Lichtenstein (2) published in 1942 and based on sampling in 1938–1939 the mean value for body weight in the present investigation is 1–3 kg higher in all age groups The group means for height are also 1–3 cm higher (Table 7 *a*) For the 8 year-old group the values are in good agreement with current height and weight data from a longitudinal Swedish study by Karlberg et al (12) In Table 7 *a* these values are given for comparison The 4-year-olds in Vasterbotten that is in the city of Umeå were somewhat taller than the children in Karlberg s study in Stockholm The difference probably reflects the secular trend since the 4 year-olds in Umeå were studied 7–8 years later For the 13 year-olds there were small differences in weight and height between the two investigations However the ages of the two materials for the 13 year-olds are not exactly comparable This must be born in mind when evaluating the differences in body measurements It is striking that the differences between Karlberg s Stockholm children and the children in Vasterbotten are so small

For all age groups the mean skinfold measurements were greater in the Stockholm material than in the children in Vasterbotten (Table 7 *b*) The differences are even greater if the Stockholm children are compared only with the rural children in the present study These differences are probably due to a number of factors such as diet activity and socio-economic status An analysis of the possible relation between the investigated children s general and the oral health status food habits and socio economic situation has been carried out and the results are described by Samuelson et al (21)

In regard to the health status of the children there was a notably high frequency of upper respiratory infections in all age groups Although the children were in generally good health upper respiratory infections were present at the time of the examination or had recently been present This was reflected in the elevated microsedimentation rates especially among the preschool children In the clinical examination as well it was found that the 4 year-olds had a higher frequency of symptoms of infection and enlargement of the superficial lymph nodes in the neck than did the other age groups

The ESR values found by Mellbin (16) in Lapp children in the northern part of Sweden were of the same order of magnitude in the school children studied as in the present material Subclinical infections were present in his material just as in the present study

Hofvander (9) in his investigation of privileged children in Addis Ababa aged 1–14 years found a high frequency of infections which were not serious in nature but which were reflected in elevated ESR values In Hofvander s material the mean ESR for 1 to 4-year old children was 17 5 mm for 5 to 9 year old children 13 3 mm and for 10 to 14-year olds 12 7 mm The ESR was higher than 20

Table 7*a* *Swedish investigations on weight and height in corresponding age groups*

	Sex	No	4 years 5 months Weight Mean	S D	Height Mean	S D	No	8 years 4 months Weight Mean	S D	Height Mean	S D	No	13 years 4 months Weight Mean	S D	Height Mean	S D
Broman Dahlberg & Lichtenstein (1942)[a]	Boys		17 2	1 9	104 0	4 3		25 9	3 5	128 5	5 5		42 7	7 8	153 6	7 9
	Girls		16 6	2 0	102 8	4 4		25 1	3 7	127 0	5 7		44 3	7 6	155 0	6 9
Samuelson (1971)	Boys	99	18 1	2 4	107 0	4 7	297	26 9	4 3	129 6	5 5	305	45 3	8 9	157 0	8 2
	Girls	99	17 9	2 4	106 5	4 2	286	26 8	4 6	129 3	5 6	315	47 8	8 0	158 0	6 7
			Interpolated values				*Interpolated values*					*Values age 13 0 years*				
Karlberg et al (1971)	Boys	113	17 8	2 3	106 3	4 0	112	27 0	4 2	130 6	5 4	93[b]	43 4	8 4	156 3	7 5
	Girls	83	17 6	2 2	105 4	4 3	82	27 1	4 6	129 2	5 8	64[b]	47 0	8 8	157 8	7 2

[a] 3 6–4 5 years 7 6–8 5 years 12 6–13 5 years
[b] The children who had attained the age of 13 0 years in Nov 1970

lished, but is not unlikely, that there were differences in the early feeding of the urban children and the children in the mountain foreland The current nutritional situation, which was generally the same in all areas, cannot have been decisive in this respect Although the genetic heterogeneity of the population of Vasterbotten is well documented (1) there is no conclusive evidence that the somatic differences between areas found in this study are due to genetic factors

In the present study it has been shown that differences in socio-economic conditions play a role in the explanation of the differences in body height found between the areas (21) Mellbin (16) in his study of children of nomadic Swedish Lapps in the northern part of Sweden, also found geographical differences in children s growth and development The population in the northernmost area investigated had been more isolated and lived under poorer socio-economic conditions Mellbin found that the Lapp children in the northernmost area were shorter than those farther south It was assumed that this difference was mainly due to differences in socio economic conditions, although genetic factors were discussed as having a certain amount of importance Children born 1950–1953 were found to be taller for their ages than those born 1942–1946 This difference was most pronounced in the far north In all parts of the world the importance of socio

Table 7*b* *Swedish investigations on skinfold thickness in corresponding age groups*

	Sex	No	4 years 5 months Triceps Mean	S D	Subscapular Mean	S D	No	8 years 4 months Triceps Mean	S D	Subscapular Mean	S D	No	13 years 4 months Triceps Mean	S D	Subscapular Mean	S D
Samuelson (1971)	Boys	88	8 6	1 9	5 0	1 2	297	8 2	2 6	5 1	2 1	305	8 9	3 4	6 4	3 3
	Girls	63	9 4	1 8	5 5	1 3	286	10 1	3 3	6 5	3 5	315	12 1	4 4	8 6	4 0
			Interpolated values					*Interpolated values*					*Values age 13 0 years*			
Karlberg et al (1971)	Boys	111	9 9	1 7	5 4	1 5	112	9 2	3 3	5 0	1 8	90[a]	10 0	4 1	7 0	4 5
	Girls	81	10 3	1 8	6 3	1 6	80	11 2	3 2	6 6	3 3	60[a]	12 4	3 2	9 2	4 1

[a] The children who had attained the age of 13 0 years in Nov 1970

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mm in 28 1% of the 1 to 4-year-olds, in 15 4% of the 5 to 9 year olds and in 12 3% of the 10 to 14-year olds Both the mean values and the frequencies of ESR values above 20 mm are in fairly good agreement with the findings in the present study

For the 8-year-olds, the difference in mean ESR's between the inland area and the mountain foreland was very small It was probably due to a somewhat higher incidence of upper respiratory infections in the mountain foreland during the 4 month period of investigation

The other findings in the clinical investigation were less remarkable The frequency of eczema for instance in this study was found to be 6 1% In a health screening program of 4 year olds resident in another county of Sweden Vuille (25) found about the same frequency (6 0%) All the children were receiving preventive health care through child health centres and school health services

In the haematological investigation of the children (20), the incidence of iron deficiency anaemia was found to be very low in all age groups Only two 13-year-old girls were found to have depleted iron stores, mainly because of heavy losses of iron in menstruation

In summary, the health and nutritional status of both the rural and urban children were found to be good This no doubt reflects the availability of an abundance of foods of various kinds in the areas studied the increasingly comprehensive nature of preventive health services, and the successive improvement in socio economic conditions

SUMMARY

The health and nutritional status of 1 401 children aged 4 8 and 13 years in one urban area and two rural areas of a northern Swedish county were studied

In none of the age groups was there any undernutrition Anthropometrical measurements reflected the good nutritional status, with height and weight generally conforming to current values of a Swedish urban population The girls had significantly thicker skinfolds than the boys The children in the city of Umea had thicker skinfolds than the children in the mountain foreland and were also significantly taller

There was a rather high frequency of subclinical upper respiratory infections among the children, especially among the 4 year olds

ACKNOWLEDGEMENTS

My thanks are due to Prof P Karlberg Gothenburg and collaborators who placed their unpublished results of anthropometrical measurements in Stockholm at my disposal

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Table 1 *Results of the screening study among newborn babies*

Total no of children tested	Blood phenylalanine		
	<4 mg/ 100 ml	4–6 mg/ 100 ml	>6 mg/ 100 ml
71 135	*71 111*	24	0

90% of which were idiots or imbeciles) have thus far been screened with the bacterial inhibition test With two cases of PKU among them a prevalence of 0 54 per 1 000 is obtained The average incidence of PKU among institutionalized mentally retarded patients in 12 countries was 0 64 % (6) and the results from the Scandinavian countries fall very close to that figure The difference between these prevalences is significant at the level $p<0\,001$ as tested with the Poisson s distribution That the incidence of PKU among the Finnish newborn does not exceed one per 100 000 is well established The Finns thus fall into the same category as the American blacks (1)

Finland with her total population of 4 800 000 has a rather remote location in Northern Europe The immigration rate is very low and the main population stock comes from the East Baltic and Nordic races The ethnic background thus differs from that of the other Scandinavian countries The closest ethnic relatives the Fenno-Ugrians, mostly live in the Soviet Union from where no published data on PKU are available No cases of PKU have been found in Estonia (3) but a screening study among the Hungarians who also have ethnic ties with the Finns revealed three cases of PKU among 20 000 newborns (7) The Finns obviously have a very special genetic background Many rare diseases such as congenital nephrosis selective vitamin B_{12} malabsorption congenital chloridorrhoea protein intolerance with lysinuria and aspartylglucosaminuria have been found more frequently in Finland than in the rest of the world On the other hand some other diseases such as cystic fibrosis galactosaemia and PKU are much less common than in other European countries

Some error may have been introduced into the present screening system because a number of premature and sick infants escaped the sampling However it is not known that the birth weight of PKU patients is lower than that of other infants nor is there any reason to expect them to have more difficulty during the neonatal period than normal babies As advocated by Hsia & Dobson (8) in all cases the Guthrie test was performed later than 48 hours and only after 24 hours of feeding with proteins Therefore the results obtained were not due to lack of feeding

The screening of newborn babies was stopped during May 1970 because of the extremely low incidence of the disease

SUMMARY

Guthrie s bacterial inhibition test was applied to 71 135 newborn infants from different parts of Finland No cases of PKU were diagnosed In addition 3 685 mentally retarded patients mostly idiots and imbeciles have been screened in Finland with the same method With two cases of PKU among them a prevalence of 0 54 per 1 000 is obtained The incidence of the disease among the newborn is estimated to be less than one per 100 000

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The authors wish to express their gratitude to the personnel of all hospitals that participated in the screening programme The financial aid provided by Mannerheimin Lastensuojeluliitto is greatly appreciated.

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THE INCIDENCE OF PKU IN FINLAND

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From the Children s Hospital Department of Neurology and Institute of Sero Bacteriology University of Helsinki Helsinki Finland

The incidence of phenylketonuria (PKU) among newborn babies is approximately one per 10 000 in most Western countries It is racially determined, and some very low figures have also been reported e g among the American blacks (1) and among the Ashkenazi Jews (2) The incidence was assumed to be of the same order in Finland as in the Scandinavian countries However, only three cases had been diagnosed through all time by 1966 and this led to the suggestion that PKU might be an exceptionally rare disease in Finland (3) To estimate the true incidence of this disease, wide range screening of the newborn was initiated In addition a number of institutionalized patients were studied This paper reports the results of these studies

MATERIAL AND METHODS

Twelve obstetric hospitals in various parts of the country took part in the project After careful instruction of the nurses and laboratory technicians the first blood specimens taken by heel puncture were sent to the central laboratory on Nov 11 1966 By April 30 1970 a total of 71 135 newborn infants had been tested representing about one third of the babies born during the same period in Finland Glucose solution was given to all babies 4 to 24 (average 12) hours and breast milk 24 hours after birth Mother s milk was also given when breast feeding was not possible except in a few instances when cow s milk was given The blood sample was taken a minimum of 96 hours after the birth Premature or sick children who had been transferred to other wards or hospitals were excluded from the series In addition to the newborn babies a total of 1 595 patients at various institutions for the mentally retarded were also screened The patients were taken at random but appr 90 were profoundly severely or moderately retarded (idiots and imbeciles) Serum phenylalanine was determined by the conventional bacterial inhibition test (4) Samples with a known elevated phenylalanine level were occasionally sent to the laboratory as routine specimens for control purposes The reliability of the method was verified with a fluorimetric procedure (5)

RESULTS

The results of the screening programme among the 71 135 newborn babies are presented in Table 1 No case of permanent hyperphenylalaninaemia was discovered Blood phenylalanine levels of between 4 mg/100 ml and 6 mg/100 ml were observed in 24 babies, but the result was normal in every instance when a control sample was tested

No cases of PKU were found among the 1 595 institutionalized mentally retarded patients

DISCUSSION

Because no cases of PKU were detected among the newborn the figures obtained from the screening of the mentally retarded patients must be utilized in an estimation of the prevalence of the disease in Finland A total of 2 090 patients were screened earlier, among whom two cases of PKU were detected (3) and in addition 1 595 cases were examined now, so that a total number of 3 685 institutionalized mentally retarded patients (appr

RENAL FUNCTION TESTS IN NONACUTE RECURRENT URINARY TRACT INFECTIONS IN CHILDREN

U BERG

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children with recurrent urinary tract infec ns examination of renal function is of ut ost importance in following the natural pro ession of the disease Clearance studies are esently considered to be the most valid chniques for determining renal function This pe of studies must be restricted for several asons Most patients with recurrent urinary act infections will therefore be followed by ch blood and urine analyses which will only ve qualitative estimates of either glomerular r tubular function In the present study the sults from various clearance studies have en compared with the results of blood and ine analyses The correlation between glo- erular and tubular function has also been udied

MATERIAL

5 patients 4 boys and 31 girls aged 3 /12 to 17 /12 ears have been studied All the patients had histories f recurrent urinary tract infections confirmed by ine cultures. They were all without any clinical or acteriological signs of infection the last two months rior to the presented studies The results from the earance studies hav been given in previous reports 2, 3 5 6) The blood and urine tests were obtained n th same period of time (within 2 weeks) as the earance studies

METHODS

Glomerular filt ation rate

Blood tests For the analysis of serum creatinine and blood urea nitrogen blood samples were withdrawn in the morning with the patients fasting Serum creatinine was analysed in venous blood samples 5 ml blood was required Blood urea nitrogen was analysed in capillary blood samples 0 1 ml blood was required

Clearance studies Glomerular filtration rate was determined by the clearance of inulin (14) For this purpose a continuous infusion of 10 % inulin (Laevasar-Gesellschaft) 0 001 g/min/kg body weight was given following the prime dose of 0 05 g/kg b w The continuous infusion was begun 60 min before the first urine collection period was started in order to obtain saturation of extracellular volume with inulin For urine sampling a double lumen polyethylene catheter was used enabling a continuous suction. Blood samples were taken in the middle of each urine sampling period from an indwelling needle in a peripheral vein

Concentrating capacity

Fluid deprivation tests One to three urine samples obtained by spontaneous voiding were taken after the patients had been deprived of fluid and food for 19 hours In some of the patients the fluid deprivation test was repeated but at this time with the intramuscular injection of Pitressin tannate® (Parke & Davis) in a dosage of 0 5 pressor units per 6 kg body weight (16) The urine specimens were analysed for osmolality The maximal urine osmolality was used for further analysis

Clearance studies Following 19 hours of water deprivation 2–3 urine collection periods were obtained each of about 45 min Urine samples were obtained by continuous suction from a double lumen polyethylene catheter inserted in the bladder Venous blood samples were withdrawn in the middle of each urine collection period The studies were performed during the continuous infusion of inulin enabling simultaneous determination of filtration rate Urine and blood samples were analysed for osmolality and inulin

Diluting capacity

Induction of water diuresis The aim of this procedure was to eliminate the effect of endogenous

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RENAL FUNCTION TESTS IN NONACUTE RECURRENT URINARY TRACT INFECTIONS IN CHILDREN

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In children with recurrent urinary tract infections examination of renal function is of ut most importance in following the natural progression of the disease Clearance studies are presently considered to be the most valid techniques for determining renal function This type of studies must be restricted for several reasons Most patients with recurrent urinary tract infections will therefore be followed by such blood and urine analyses which will only give qualitative estimates of either glomerular or tubular function In the present study the results from various clearance studies have been compared with the results of blood and urine analyses The correlation between glomerular and tubular function has also been studied.

MATERIAL

35 patients, 4 boys and 31 girls aged 3 /₁₂ to 17 /₁₂ years have been studied All the patients had histories of recurrent urinary tract infections confirmed by urine cultures. They were all without any clinical or bacteriological signs of infection the last two months prior to the presented studies The results from the clearance studies have been given in previous reports (2, 3 5 6) The blood and urine tests were obtained at the same period of time (within 2 weeks) as the clearance studies

METHODS

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Clearance studies Following 19 hours of water deprivation 2–3 urine collection periods were obtained each of about 45 min Urine samples were obtained by continuous suction from a double lumen polyethylene catheter inserted in the bladder Venous blood samples were withdrawn in the middle of each urine collection period The studies were performed during the continuous infusion of inulin enabling simultaneous determination of filtration rate Urine and blood samples were analysed for osmolality and inulin

Diluting capacity

Induction of water diuresis The aim of this procedure was to eliminate the effect of endogenous

Supported by the Swedish Medical Research Council Grant B67 19X 2049-01A.

antidiuretic hormone (12) For this purpose the patients were asked to drink water in an amount corresponding to 2–2 5% of body weight in an hour and thereafter in amounts corresponding to 0 5 of body weight every 30 min When the diuresis was constant which generally occurred 90 min after the intake of fluid was started urine collection was begun

Test of minimum urine osmolality Following the induction of water diuresis i e when the diuresis was constant 2–3 spontaneously voided urine samples were obtained The specimens were analysed for osmolality The means of the determinations obtained were used for further analysis

Clearance studies Following the induction of water diuresis i e when the diuresis was constant 3–4 urine collection periods each of about 15 min were obtained Urine samples were obtained by continuous suction from a double lumen polyethylene catheter inserted in the bladder Venous blood samples were withdrawn in the middle of each urine collection period The studies were performed during the continuous infusion of inulin Thus simultaneous determinations of the filtration rate could be obtained

Sodium homeostasis

Blood tests Venous blood samples were withdrawn in the morning while the patients were fasting The blood samples were analysed for sodium

Urine tests The urine samples were those obtained during the fluid deprivation tests The urine specimens were analysed for sodium

Clearance studies Clearance studies were performed during hydropenia i e after 19 hours of water deprivation The studies were performed during the continuous infusion of inulin thus allowing simultaneous determinations of filtration rate Urine samples were obtained by continuous suction from a double lumen polyethylene catheter inserted in the bladder Venous blood samples were withdrawn in the middle of each urine collection period

Renal regulation of acid base balance

Blood tests Prewarmed capillary blood samples were taken in the morning for the analysis of standard bicarbonate

Short time ammonium chloride test (8) The study was started after a standard breakfast meal In order to keep a constant urine flow rate the patients were given water in an amount of 0 1–0 2 ml/min/kg body weight The forced fluid intake was started 2–4 hours before the intake of ammonium chloride Urine was collected hourly by spontaneous voiding Following control sampling of blood and urine ammonium chloride was given in the amount of 150 mEq/m² body surface After the administration of the ammonium chloride another five urine samples were collected and two to three blood samples were taken 2 3 and 5 hours after the administration Immediately after voiding the urine samples were withdrawn and kept anaerobically on ice pH analysis were carried out within 15 min after voiding Prewarmed capillary blood samples were taken and analysed for actual pH in samples equilibrated with 4 and 8 CO_2 By plotting those data on a Siggaard Andersen curve nomogram P_{CO_2} standard bicarbonate and total CO_2 could be obtained (13)

Analytical methods

Serum creatinine was determined as true creatinine with the Jaffe reaction after adsorption on to Lloyds reagent Blood urea nitrogen was determined by analysing for ammonia colorimetrically before and after the action of urease (9) Inulin in blood and urine was determined according to the method of Heyrovsky (11) Osmolality in blood and urine was determined cryoscopically with a Knauer osmometer Sodium in blood and urine was determined with a flame photometer (Eppendorf) pH in urine was determined on a pH meter 26 (Radiometer) pH in blood was determined on a pH meter 27 (Radiometer)

Calculations

The clearance of inulin was calculated from th formula

$$C_{In} = \frac{U_{In} \times V}{B_{In}}$$

where U_{In} and B_{In} stand for the concentrations of inulin in urine and blood respectively V stands for the diuresis

Free water reabsorption (T_{H_2O}) was calculated according to the formula

$$T_{H_2O} = C_{osm} - V$$

where C_{osm} is calculated from th formula

$$C_{osm} = \frac{U_{osm} \times V}{B_{osm}}$$

where U_{osm} and B_{osm} stand for the osmolality in urine and blood respectively V see above

Free water clearance (C_{H_2O}) is calculated from the formula

$$C_{H_2O} = V - C_{osm}$$

Symbols see above

Fractional sodium excretion (C_{Na}/C_{In}) is calculated from the formula

$$C_{Na}/C_{In} = \frac{U_{Na} \times V \times 100}{B_{Na} \times C_{In}}$$

where U_{Na} and B_{Na} stand for th concentrations of sodium in urine and blood respectively V and C_{In} see above

Total CO_2 concentration = actual bicarbonate concentration + 0 0306 × actual P_{CO_2} Student s t test has been used for th statistical analysis

RESULTS AND COMMENTS

Glomerular filtration rate

Two blood tests are used for screening of glomerular filtration rate, namely serum creatinine concentration and blood urea nitrogen

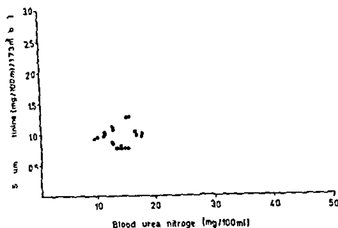


Fig 1 Relationship between blood urea nitrogen concentration (mg/100 ml) and serum creatinine concentration (mg/100 ml/1 73 m b.s) 41 observations in 32 patients are included

concentration The advantage with the determination of serum creatinine is its relative stability despite variations in the protein intake (4) The analytical method is however time consuming and generally venous blood samples are needed for the analysis The advantage with blood urea nitrogen determination is the simple method of analysis and the small amounts of blood required The disadvantages with blood urea nitrogen determination are however the variations of blood urea nitrogen concentration with protein intake and water content of the body (1 15) Fig 1 demonstrates the relationship between blood urea nitrogen and serum creatinine concentration Since serum creatinine is related in a linear fashion to body surface (7) the values have been corrected for 1 73 m b s When serum creatinine is below 1 3 mg/100 ml which is considered to be the upper limit of normal and blood urea nitrogen concentration is below 20 mg/100 ml there is no apparent relationship between serum creatinine and blood urea nitrogen In fact variations between serum creatinine concentration and blood urea nitrogen concentration are even found in each individual when followed continuously as demonstrated in Fig 2 When blood urea nitrogen concentration exceeds what is considered to be upper limit of normal (20 mg/100 ml) the correlation between blood urea nitrogen concentration and serum creatinine concentration is fairly good

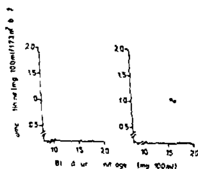


Fig Relationship between blood urea nitrogen concentration (mg/100 ml) and serum creatinine concentration (mg 100 ml/1 73 m b.s.) in two patients on 7 and 6 different occasions respectively

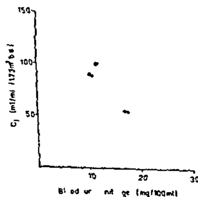


Fig 3 Relationship between blood urea nitrogen concentration (mg/100 ml) and clearance of inulin (C ml/min/1 73 m b s) during hydropenia 27 observations in 24 patients are included

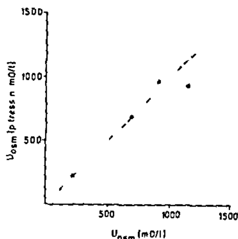


Fig 4 Relationship between urine osmolality (U_{osm} mO/l) after *19* hours of water deprivation and urine osmolality after pitressin tannate was injected followed by *19* hours of water deprivation 11 patients are included The dashed line indicates the isosmotic line

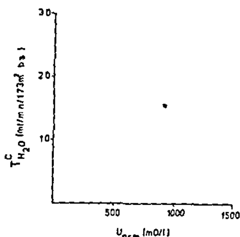


Fig 5 Relationship between urine osmolality (U_{osm} mO/l) after 19 hours of water deprivation and free water reabsorption ($T^c_{H_2O}$ ml/min/1 73 m² b s) 25 observations in 21 patients are included

(correlation coefficient 0 97, highly significant) *Thus no difference between the two methods* can be noticed Presently therefore blood urea nitrogen determination can be recommended in pediatric praxis because the small amount of blood required for the analysis Fig 3 demonstrates the relationship between blood urea nitrogen concentration and the clearance of *inulin during hydropenia The state of hydro*penia was chosen because the blood test was taken in the morning with the patients fasting An increase in blood urea nitrogen concentration is generally not noted until the glomerular filtration rate is reduced below 50 ml/min/1 73 m² b s This is in accordance with the results of de Wardener (15) who studied the relationship between clearance of creatinine and blood urea nitrogen in adults A normal value of blood urea nitrogen will thus only mean that the filtration rate is above 50 ml/min/1 73 m b s

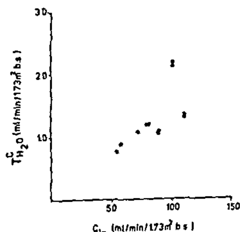


Fig 6 Relationship between glomerular filtration rate (C_{In} ml/min/1 73 m² b s) during hydropenia and free water reabsorption ($T^c_{H_2O}$ ml/min/1 73 m² b s) 25 observations in 21 patients are included

Concentrating capacity

Fig 4 demonstrates the relationship between urine osmolality after 19 hours of fluid deprivation and *urine osmolality after pitressin tan*nate was given in combination with fluid deprivation The time interval between the two tests does not exceed a week The scatter of the results is fairly large but there is no typical trend in the deviations Fig 5 demonstrates the relationship between urine osmolality after *19 hours of fluid* deprivation and free water reabsorption There is a highly significant positive relationship between free water reabsorption and maximal urine osmolality correlation coefficient 0 63 It should be noted that maximal urine osmolality and free water reabsorption are measurements of two related but not identical determinants of concentrating capacity *The maximal urine* osmolality is a measurement of the tonicity of the renal medulla

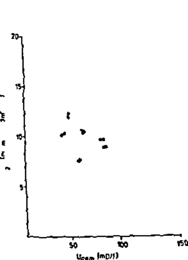


Fig 7 Relationship between minimum urine osmolality (U_{osm} mO/l) and free water clearence (C_{H_2O} ml/min/1 73 m bs) 37 observations in 28 patients are included

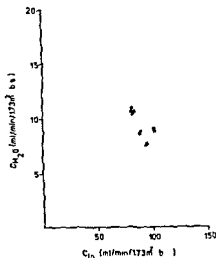


Fig 8 Relationship between glomerular filtration rate (C_i ml/min/1 73 m bs) during water diuresis and free water clearence (C_{H_2O} ml/min/1 73 m² bs) 37 observations in 28 patients are included

and thus an index of the function of the loop of Henle Determination of free water reabsorption is a method of quantitating the principal aim of urine concentration i e fluid conservation In hydropenia the kidneys excrete a urine which is more concentrated than the blood The glomerular filtrate has the same osmotic concentration as the plasma (10) In order to concentrate the urine the renal tubules must remove a certain quantity of solute free water from the isosmotic glomerular filtrate This volume of water removed can be calculated by the difference between the volume of urine required to contain urinary solutes in an isosmotic state i e the osmolar clearance C_{osm} and the urine flow V The free water reabsorption can be defined as the volume of water reabsorbed in excess of an isosmotic equivalent of solute Free water reabsorption is thus a qualitative and a quantitative measurement of concentrating capacity By determining free water reabsorption the reserve capacity of the loop of Henle can be estimated in the limits of increased solute loading. The reserve capacity cannot be assessed by the determination of urine osmolality only For clinical purpose however determination of maximal urine osmolality is sufficient for the evaluation of the concentrating capacity provided there is no osmotic diuresis as in glucosuria Fig. 6 demonstrates the relationship between glomerular filtration rate determined by the clearance of inulin during hydropenia and free water reabsorption When filtration rate is reduced a depressed free water reabsorption is noticed The correlation coefficient is 0 75 which is highly significant Thus there is no great discrepancy between filtration rate and the tubular function dealing with concentration of urine in recurrent urinary tract infections

Diluting capacity

Fig 7 demonstrates the relationship between minimum urine osmolality and free water clearance With decreasing urine osmolality an increase in free water clearance is seen The correlation coefficient is 0 58 which is highly significant Minimum urine osmolality and free water clearance are measurements of two related but not identical determinants of diluting capacity The minimum urine osmolality is a qualitative measurement of diluting capacity and thus an index of the function of the ascending limb of Henle and the first part of distal

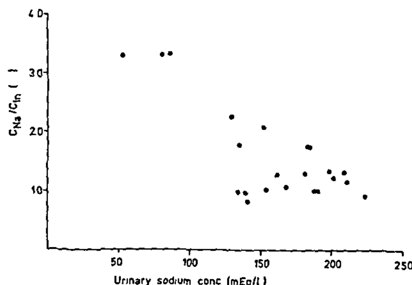


Fig 9 Relationship between urinary sodium concentration (U mEq/l) and fractional sodium excretion (C_{Na}/C_{In} %) during hydropenia 24 observations in 20 patients are included

convoluted tubule Free water clearance measures the amount of water being discharged from solutes when passing through the nephron mainly the ascending limb of Henle and the early distal tubule Thus free water clearance is both a qualitative and a quantitative measurement of the function of these parts of the nephron *In contrast to minimum urine os-*molality free water clearance can therefore be used for evaluating the reserve capacity in the ascending limb of Henle and early distal tubule For clinical purpose however urine osmolality is sufficient for evaluating the diluting capacity Fig 8 demonstrates the relationship between glomerular filtration rate determined by the clearance of inulin during water diuresis and free water clearance There is a highly significant positive relationship between free water clearance and glomerular filtration rate correlation coefficient 0 65 This indicates that there is a rather homogenous reduction of the glomerular and distal tubular functions in recurrent urinary tract infections in children

Sodium homeostasis

Serum sodium concentration was within normal limits, 133–146 mEq/l, in all patients studied Fractional sodium excretion, however, which is normally below 1 8% (2) was in the range of 2 1 to 3 5% in 5 patients This increase in fractional sodium excretion did apparently not result in hyponatremia Fig 9 demonstrates the relationship between urine sodium concentration and fractional sodium excretion The values were obtained during hydropenia With increasing fractional sodium excretion there is a decrease in urinary sodium concentration Thus in contrast to what has generally been considered determination of urine sodium concentration might be a valuable tool in the assessment of renal function This assumes that urinary sodium concentration is determined during a controlled diuretic state e g maximal hydropenia It is therefore suggested that urine sodium concentration should be routinely examined during a conventional fluid deprivation test The mean urine sodium concentration during hydropenia in patients with filtration rates above 80 ml/min/1 73 m² b s and fractional sodium excretion below 1 8% is 179 ± 56 mEq/l (mean value ± 2 S D) A deviation in urine sodium concentration below −2 S D, i e 123 mEq/l then strongly suggests increased fractional sodium excretion Fig 10 demonstrates the relationship between glomerular filtration rate and fractional sodium excretion during hydropenia With decreasing filtration rates there is an increase in fractional sodium excretion This might be a sign of a homogeneous reduction of glomerular and tubular functions but reports from this laboratory have indicated that the relationship between

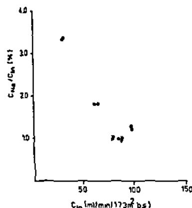


Fig 10 Relationship between glomerular filtration rate (C ml/min/1 73 m b s) and fractional sodium excretion (C_N /C_I) during hydropenia 24 observations in 0 patients are included

filtered load of sodium and reabsorbed sodium might rather be a physiologically induced reset of glomerular tubular balance for sodium (3)

Renal regulation of acid base balance

The contribution of the kidneys to the maintenance of the acid base balance is accomplished by the secretion of hydrogen ions These hydrogen ions either participate in the reabsorption of bicarbonate or are excreted in the form of ammonium ions or titratable acids Fig 11 demonstrates the relationship between basal standard bicarbonate concentration and the urine pH value when the blood total CO concentration by an oral acid load has been reduced to 20 mM/l In healthy children the urine pH at this blood total CO concentration of 20 mM/l is below 5 5 No good relationship can be found between the urine pH at the total CO concentration of 20 mM/l and the standard bicarbonate concentration in children with recurrent urinary tract infections Thus 7 of 12 patients with normal standard bicarbonate levels had signs of some defect in urinary acidifying mechanisms i e urine pH value above 5 5 at the total CO concentration of 20 mM/l Previously we have shown that a defect in the reabsorption of filtered bicarbonate is most likely the most prominent change in urinary acidifying mechanisms (6) Since the majority of patients do not show any signs of acidosis it is probable that the degree of the defect in bicarbonate reabsorption varies from nephron to nephron and that some of the nephrons are still capable of a practically normal function in respect to bicarbonate reabsorption This would explain why the urinary bicarbonate loss in most patients is sufficiently small not to result in renal acidosis

Fig 12 demonstrates the relationship between glomerular filtration rate during water diuresis and the urine pH at a total CO concentration of 20 mM/l It is highly noticeable

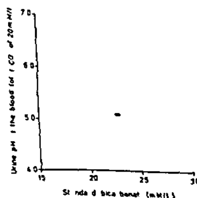


Fig 11 Relationship between the basal standard bicarbonate concentration (mM/l) and the estimated urine pH at the blood total CO concentration of 0 mM l 16 patients are included

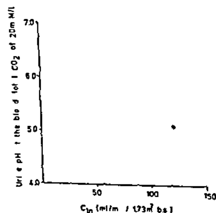


Fig 12 Relationship between glomerular filtration rate (C ml min/1 73 m b s) during water diuresis and the estimated urine pH at the total CO concentration of 20 mM/l 16 patients are included

that 5 of 9 patients with normal filtration rates demonstrate a defect in urinary acidifying mechanisms

Thus determination of renal acidifying capacity might be the most sensitive tool in the assessment of renal function in recurrent urinary tract infections in children Again it should be postulated that no quantitative estimation of renal hydrogen ion secretion could be done with the short time ammonium chloride test

CONCLUSION

Previous studies from this laboratory have shown that changes in renal function in children with recurrent urinary tract infections practically always occur when renal parenchymal changes can be demonstrated with an intra venous pyelogram On the basis of the present results it is recommended that these patients at least yearly are followed by the following tests blood urea concentration, fluid deprivation test with determination of urine osmolality and of urine sodium concentration, water load test with determination of urine osmolality and a short time ammonium chloride test If possible a clearance study with the determination of the glomerular filtration rate should also be carried out

SUMMARY

The clinical value of various renal function tests in recurrent urinary tract infections in children has been examined The study also includes an evaluation of glomerular capacity versus tubular capacity in recurrent urinary tract infections There was no good correlation between blood urea nitrogen concentration and serum creatinine concentration within the normal limits for these two parameters When blood urea nitrogen concentration and/or serum creatinine concentration were elevated a highly significant correlation between the two parameters was found When relating blood urea nitrogen concentration to the clearance of inulin an elevation of blood urea nitrogen concentration was not found until the filtration rate was below 50 ml/min/1 73 m² b s The tubular functions were examined by studies of (*a*) the concentrating capacity, (*b*) the diluting capacity, (*c*) sodium reabsorption and (*d*) renal acid base regulation The concentrating capacity was determined by two screening tests, maximal urine osmolality after 19 hours of fluid and food deprivation with or without injection of pitressin tannate, and by free water reabsorption The administration of exogenous pitressin had no significant effect on maximal urine osmolality There was a highly significant correlation between free water reabsorption and maximal urine osmolality A highly significant correlation was also found between free water reabsorption and glomerular filtration rate Diluting capacity was evaluated by determining minimum urine osmolality and free water clearance A highly significant correlation between minimum urine osmolality and free water clearance was obtained There was a good correlation between free water clearance and glomerular filtration rate When sodium reabsorption is depressed below normal, urine sodium concentration during hydropenia is abnormally low A normal standard bicarbonate level does not exclude a defect in renal acidifying mechanisms A defect renal acidifying capacity could be found even in patients with normal filtration rates indicating that renal acidifying capacity might be the first sign of renal damage in children with recurrent urinary tract infections

ACKNOWLEDGEMENT

The author is greatly indebted to Miss Lill Britt Jonsson and Miss Eivor Sundqvist for excellent technical assistance

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Table 1 *Plasma growth hormone and growth response to human growth hormone*

Pat	Sex	Age (y)	Max GH level during ITT mμg/ml	Max GH increase during ITT mμg/ml	Growth response to human growth hormone: Growth velocity 6 months prior to therapy cm/yr	Growth velocity first 6 months on therapy cm/yr
Primordial growth retardation						
Sch D	♂	7	13 5	+ 6 9	5 4	7 3
M Ch	♂	7½	29 0	+26 0	4 8	6 1
A A	♂	10	32 0	+32 0	4 1	10 0[a]
Hypopituitary growth retardation						
C M	♂	7	1 6	+ 1 1	3 4	10 2
W D	♂	11	0 9	+ 0.3	2 7	10 6[b]
Co M	♂	15	0 8	0	2 4	13 1
Co P	♂	16	0 7	− 0 1	3 5	10 4

ITT Insulin tolerance test intravenously 4 Units Actrapid Novo/m²
[a] *Growth spurt due to spontaneous onset of puberty*
[b] Patient receiving thyroid in addition to growth hormone because of documented TSH deficiency

ministration of 0 33 g glucose/kg bodyweight as a 40% solution over a one minute period (14)

On a separate day a one hour glucose infusion test (modified method of Cerasi & Luft (4)) was performed as follows It was started with a priming dose calculated to reach an initial glucose level of 260 mg/100 ml Immediately after the priming dose a glucose infusion was administered by a syringe type constant pump (Braun Melsungen) at a flow rate of 120 ml/hour and at a concentration calculated from the K value in order to obtain a constant glucose level at approximately 260 mg 100 ml during a 60 min period (mean dosage 21 2 mg/kg/min range 15 8-28 8) After 60 min the glucose infusion was replaced by a normal saline infusion at the same rate Blood was drawn at −15 0 +15 +30 +45 +60 +90 +120 +150 and −180 min through an indwelling plastic or siliconized needle and was immediately chilled in ice water Glucose was determined by a glucoseoxidase method (21) free fatty acids by a titrimetric method (24) growth hormone by a double antibody radioimmunological method (28) from plasma Insulin was assessed by a double antibody radioimmunological method (11 17) from serum

During one week prior to the examination the usual weekly dosage was divided into daily administrations (e g 1 43 mg/m /injection) and on the day of the test the dose was given exactly 60 min prior to the beginning of the glucose infusion

The results were plotted on millimeter paper the

Table 2 *Influence of growth hormone on glucose disappearance rate (K value)*

	Prior to therapy	6 months	12 months	18 months	27 months
Primordial growth retardation					
Sch D	1.66	1 82			
M Ch	2.16	3 64			
A A	2.16	3 04			
Hypopituitary growth retardation					
C M	1.48	2.48	2.57	3 14	2 56
W D	3 4	2.31	2.40	2.48	2 16
					1 78[a]
Co M			1 54	1.54	
Co P			2.10	1 87	
Normal children					
n = 20 age 3-14 yrs	2.04 ± 0 31 (S D)				

[a] On 1.2 mg HGH day

Acta Pædiat Scand 60 678–684, 1971

INFLUENCE OF LONG TERM GROWTH HORMONE THERAPY ON GLUCOSE TOLERANCE AND INSULIN SECRETION

K A ZUPPINGER and E E JOSS

From the Department of Paediatrics University of Bern Bern Switzerland

Growth rate in hypopituitary growth retardation is successfully accelerated by the administration of human growth hormone (HGH) (19 20 23, 25) Since this treatment has to be continued for a prolonged period of time, it is important to take into consideration all its possible side effects

The diabetogenic effect of growth hormone (GH) was first demonstrated by Young in 1937 (26) dogs receiving injections of extracts of anterior pituitary glands developed permanent so called metahypophyseal diabetes Endogenous overproduction of GH in acromegaly is frequently associated with a diabetic condition (6 12) which is characterized by a decreased glucose tolerance in the presence of hyperinsulinism (1, 5 7 8) Exogenous HGH in a dosage which is probably well above the physiological range was shown to produce similar derangements of carbohydrate metabolism in healthy humans (16) as well as in panhypopituitarism (15)

The dosage of HGH commonly used in the treatment of hypopituitary growth retardation is assumed to be physiological on a purely empirical basis Furthermore, for practical reasons the weekly HGH dosage is usually given in two or three injections only Such a schedule necessarily leads to markedly elevated plasma levels, which probably remain pathologically elevated for several hours after intramuscular administration (18) A longitudinal study of carbohydrate metabolism during HGH substitution therapy is therefore of interest

In the present investigation the effect of chronic administration of HGH (Raben) on glucose mediated insulin release and plasma free fatty acid concentration was studied repeatedly in three children with primordial growth retardation and in four children with documented GH deficiency

This work was supported by the Swiss National Science Foundation grant no 4588

PATIENTS

The diagnosis in the three patients with primordial growth retardation (M Ch Sch D A A) and the four with hypopituitary growth retardation (C M W D Co M Co P) was based on radioimmunological determination of GH in plasma during insulin tolerance tests The maximal rise of GH is given in Table 1 Further details and the clinical findings are reported in a separate communication (13) The investigation was carried out repeatedly over periods of 6 to 27 months after onset of HGH therapy (Raben batches 22 23) which was administered intramuscularly at a dosage of 10 mg/m²/week in three injections e g 3×3 33 mg/m²/week unless otherwise stated Growth response was slight in the children with primordial and good in those with hypopituitary growth retardation (Table 1)

For comparison a 52 year old male patient (K E) with acromegaly was studied prior to and three months after surgical removal of a pituitary adenoma[1]

METHODS

The K value for the glucose disappearance rate was determined after overnight fasting by intravenous ad

[1] This patient was kindly referred to us by PD Dr M P Konig Department of internal medicine University of Bern

Table 1 *Plasma growth hormone and growth response to human growth hormone*

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W D	3 4?	2.31	2.40	2.48	2.16
					1 78[a]
Co M			1 54	1 54	
Co P			2.10	1 87	
Normal children					
n 20 age 3 12 yrs.	2.04±0 51 (S D)				

On 1 mg HGH day

Table 3 *Influence of growth hormone on glucose, free fatty acids, and insulin during glucose infusion*

		Prior to therapy	1 week	6 months	12 months	18 months	27 months
Primordial growth retardation							
Sch D	Glucose	195 6	244 0	284 8			
	FFA	155 4	109 2	111 4			
	IRI	2 840	4 920	5 500			
M Ch	Glucose	258 4	182 4	285 0			
	FFA	110 6	109 8	59 8			
	IRI	6 220	5 820	5 960			
A A	Glucose	254 6	173 6	212 0			
	FFA	126 4	69 2	53 4			
	IRI	11 760	11 580	11 280			
Hypopituitary growth retardation							
C M	Glucose	233 6		180 4	210 8	244 6	322 2
	FFA	96 2		67 6	87 4	131 4	123 8
	IRI	4 600		4 000	4 300	7 380	4 620
W D	Glucose	292 8	302 8	252 8	236 4	194 8	270 0[a]
	FFA	73 6	127 4	136 4	62 6	100 6	85 2[a]
	IRI	5 740	7 000	4 420	4 240	4 380	10 500 [a]
Co M	Glucose				210 6	238 0	
	FFA				86 4	98 0	
	IRI				4 760	7 980	
Co P	Glucose				211 0	218 8	
	FFA				84 6	105 8	
	IRI				9 560	9 100	
Normal children							
age 3–14 yrs	Glucose	215 2±46 6 (n = 6) (mean±S D)					
	FFA	88 0 (n = 2)					
	IRI	6 260 ± 3380 (n = 6)					

The values are given in min mg ml^{-1} for glucose min μEq ml^{-1} for FFA min μU ml^{-1} for insulin
[a] On 12 mg HGH per day

area under the curve was determined planimetrically and expressed as min mg ml^{-1} for glucose min μEq ml^{-1} for free fatty acids and min μU ml^{-1} for insulin

Six normal prepuberal children aged 3-14 years (four boys and two girls) were tested as controls

RESULTS

The results are summarized in Table 2 and 3 and the findings in the normal children are given in each table

Effect on glucose tolerance

The K values were within the normal limits in the patients tested prior to therapy and there was no clear cut effect of growth hormone therapy on the K values These remained in the normal range in all treated children, even in the two patients followed for 27 months Patient W D received a six times higher dose (12 mg HGH/day) for the last five days of the study and the K value decreased only slightly from 2 16 to 1 78

Effect on free fatty acids in plasma

Neither fasting free fatty acids (FFA) nor the decreases nor the areas under the FFA curves during and after the glucose infusion test were significantly influenced by treatment with HGH (Table 3 and Fig 1) The higher HGH dosage in W D (Table 3) and the markedly increased endogenous GH level in K E prior to surgery (Fig 4) were also without effect on free fatty acids in plasma

Effect on serum insulin

In all children treated the fasting insulin concentration, the maximal rise and the area below the insulin curve during the glucose infu-

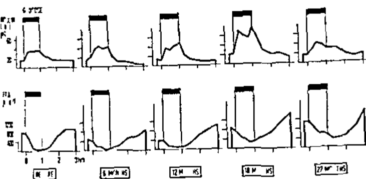


Fig 1 Influence of long term HGH (Raben) therapy (10 mg/m /week) in a patient with hypopituitary growth retardation (C M) on serum insulin and plasma free fatty acid concentration during an intravenous glucose infusion test

sion test also remained essentially normal and unaffected by HGH therapy (Table 3 Figs 1 and 2) In patient C M a distinct increase of insulin secretion seemed to arise after 18 months of HGH therapy which however was restored to the preceding level at 27 months (Fig. 1)

Administration of the higher HGH dose in W D led to plasma GH concentrations approximately four times higher (47 3 to 102 6 mμg/ml) during the test than under the ordinary dosage (11 2 to 24 0 mμg/ml) Correspondingly insulin secretion was more than twice as large under the higher dose (Fig 3 Table 3) However it failed to reach the pathologically elevated insulin secretion found preoperatively in the acromegalic patient (K E.) in whom plasma GH concentration was chronically elevated (18 8 to 39 8 mμg/ml Fig 4)

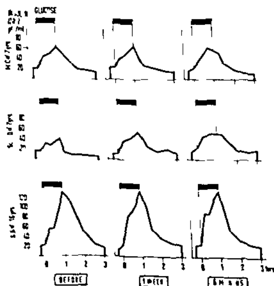


Fig 2 Influence of long term HGH (Raben) therapy (10 mg m week) in three patients with primordial growth retardation on serum insulin concentration during an intravenous glucose infusion test

DISCUSSION

The value of the interpretation of a longitudinal study depends upon the reproducibility of the parameters analyzed Repeated studies of glucose mediated insulin release under the influence of HGH in this respect are hampered by the spontaneous variability of insulin secretion *after oral glucose loads from one child to* an other but also when repeated in the same child (27) These considerations prompted us to use standardized one hour intravenous glucose infusions which seemed more appropriate for longitudinal evaluation of the effect of long term HGH therapy on the insulin releasing capacity of the pancreas Although variations from one subject to the other could not be avoided by this standardization the insulin release remained remarkably constant when repeated in the same individual as demonstrated graphically in Fig 2 Both the amount of insulin released as expressed by the area below the curve and the shape of the curve remained fairly *uniform in the* same child in spite of growth hormone therapy

Glucose tolerance and the effect of glucose infusion on insulin secretion and free fatty acids were essentially unaffected by HGH sub-

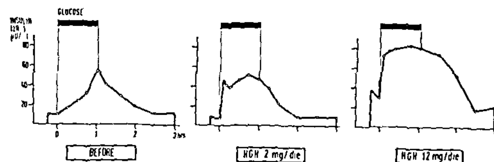


Fig 3 Influence of two different HGH doses (Raben) on serum insulin concentration during an intravenous glucose infusion test in a patient with hypopituitary growth retardation (W D)

stitution therapy in all children even after 27 months in two patients It must however be pointed out that the effect of HGH on the initial insulin release was not tested because the first blood sample was drawn after 15 min only Since this initial insulin peak after intravenous glucose administration is of very short duration in comparison to the sustained effect on serum insulin concentration by the prolonged glucose infusion the result expressed as the area under the curve is not likely to be significantly falsified by disregarding the initial insulin peak From these data therefore the conclusion may be drawn that chronic HGH therapy at the usual dosage must not be considered to be diabetogenic at least in children without diabetic predisposition

Hypopituitary growth retardation may be accompanied by hypoglycemia due to lack of GH (2) Several groups of investigators however have pointed out recently that a diabetic glucose tolerance may also be encountered in hypopituitarism (2 9 10) Our two hypopituitary patients tested prior to HGH therapy (C M and W D) showed normal glucose tolerance as judged by glucose disappearance rate The diabetic glucose tolerance found in some of the hypopituitary patients is attributed to a decrease of the glucose mediated insulin secretion frequently found in these children prior to HGH administration (9, 10) an observation which we were able to confirm in some of our other patients with GH deficiency However, the two patients of this study (C M and W D) showed normal insulin secretion prior to HGH therapy

Gold et al (10) found normalization of previously subnormal insulin secretion with concomitant normalization of glucose tolerance after onset of hormonal replacement therapy HGH therefore, at the usual dosage seems to normalize decreased insulin secretion, without producing a pathological increase of insulin release

Acute intravenous administration of 1 mg HGH (Wilhelmi) five minutes prior to glucose injection was reported to cause a significantly accelerated glucose utilization probably due to an increased insulin secretion in GH deficient subjects (9) No such effect on glucose disappearance was noted in our patients when HGH (Raben) was administered intramuscularly one hour prior to glucose injection even though the plasma GH concentrations measured in one patient (W D) were markedly elevated at the time of the test

Preliminary estimations of the daily GH production in adults were in the order of 2–5

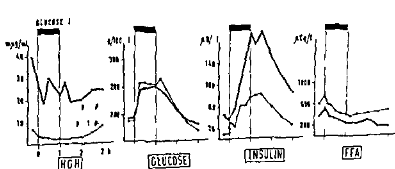


Fig 4 Effect of an intravenous glucose infusion test on serum insulin and plasma free fatty acid concentration in a patient with acromegaly (K E) before (heavy line) and three months after removal of a pituitary adenoma (thin line) Glucose tolerance remained unchanged (preoperatively K = 0 77 postoperatively K = 0 73)

mg/m (18 19) Meanwhile the study of Cameron et al (3) on the metabolic clearance rate of radioiodinated HGH revealed a multi exponential disapp arance from plasma which in fact may prevent accurate calculation of the normal daily secretion rate The dosage of HGH substitution therapy therefore will probably remain empirical Most investigators have used from 4 to 15 mg/week (19 20 22 25) *Our dose of 10 mg/m /week in three injections* conforms with the commonly used schedule Our observation of a lacking effect on insulin secretion is in agreement with the assumption that the usual dosage is roughly corresponding with the normal daily secretion rate Ten times higher doses were known to evoke a pathologically increased insulin secretion in hypopituitarism (15) The present data in one of the patients (W D) *revealed* that a six times larger dose leads to an only slightly decreased glucose tolerance It doubles however insulin secretion without affecting free fatty acid concentration Our investigation does not permit to conclude whether prolonged administration of elevated HGH doses would eventually lead to exhaustion of the pancreas with permanent diabetes mellitus It is noteworthy however that in the acromegalic patient (K E) the pathological glucose tolerance did not improve after normalization of plasma GH levels following removal of the pituitary adenoma although there was no evidence of pancreatic exhaustion since insulin secretion was normalized The reason for the continuing diabetic glucose tolerance in this patient inspite of normal GH and Insulin concentrations is unknown Yet these findings indicate that chronically elevated GH concentrations may lead to irreversible diabetes mellitus Careful adherence to the usual probably physiological HGH dosage in the treatment of hypopituitary growth retardation is therefore advocated

SUMMARY

Daily secretion of growth hormone (GH) in the normal child is unknown and may not b assessable in the near future The dosage of human growth hormone (HG) commonly used in hypopituitary growth retardation is purely empirical Growth is in general successfully accelerated by HGH but would presumably be stimulated also by higher than physiological doses Our study consisted of a longitudinal evaluation of the effects of HGH on glucose tolerance free fatty acids and immunoreactive insulin in three children with primordial growth retardation and two children with GH deficiency over six to twenty seven *months* Two additional hypopituitary children were studied twelve and eighteen months after onset of HGH therapy In all patients there was no *discernible effect on the parameters* studied at the dosage used (HGH (Raben 10 mg/m²/ week in three divided injections) This finding supports the assumption that the dose of HGH commonly used is indeed physiological in regard to carbohydrate metabolism

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STUDIES ON MATURITY IN NEWBORN INFANTS

I Birth Weight Crown–Heel Length Head Circumference and Skull Diameters in Relation to Gestational Age

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It is now well known that birth weight is a poor index of gestational age in newborn infants and that birth weight varies over a wide range for a given gestational age Treating infants of low birth weight (≤2 500 g) as a homogeneous group has been criticized from both the clinical and scientific points of view since a high proportion of low birth weight infants are born at term (10)

The normal limits for birth weights at different gestational ages are given in standard curves for the relation between these two parameters cf Hosemann (11) Lubchenko et al (15) Engstrom & Sterky (6) Sterky (20) Thomson et al (21) As a rule the relation between gestational age and crown–heel length has also been studied Few standard curves for head circumference are available (cf Hosemann (12) Lubchenco et al (16) Usher & McLean (24) Parmelee et al (18) the last mentioned is restricted to a material of low birth weight infants)

There is often a need for objective estimation of maturity or gestational age in the newborn infant The fact that birth weight has been shown to be a poor index of gestational age in newborn infants has led to the introduction of other methods for estimating age in the newborn period Postnatal radiological examination scoring of external characteristics and other methods have been used instead of anthropometric measurements However our knowledge of the value of anthropometric measurements other than birth weight and crown heel length as maturity signs is incomplete and the use of such measurements ought not to be abandoned without further study

Aims of the Present Study

1 To compare five anthropometric measurements (birth weight crown–heel length head circumference occipito-frontal diameter biparietal diameter) with respect to their correlation to gestational age
2 To present confidence limits for estimating gestational age from anthropometric measurements of the infant
3 To compare crown–heel length head circumference and skull diameters of small for gestational age infants with those of appropriate for gestational age infants

Definitions and Abbreviations Used in the Present Study

Gestational age age in days from the first day of the mother's last menstrual period until the day of birth

Postmenstrual age age in days from the first day of the mother's last menstrual period until the day of the investigation (i e gestational age plus age from birth until the investigation)

40th week days 274 to 280

SGA = small for gestational age Infant with birth weight below −2 S D in the relation between birth weight and gestational age according to Swedish standard curves (6 20)

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could not be classified according to the standard curves which do not consider gestational ages below 33 weeks (275 days)

The mean age of the mothers at delivery was 26 years SD 5.3 The youngest mother was 18 years the oldest 4? years 93 were primipara No mother had had any serious diseases during pregnancy 17 had had moderate toxemia (blood pressure above 90 mmHg diastolic in combination with albuminuria) 30 mothers had had minor bleeding at some time during pregnancy Seven infants were delivered by caesarean section the rest by vaginal delivery There were 8 breech deliveries and 2 vacuum extractions The frequencies of low fetal heart rate and low Apgar scores are seen in Tabl 3 as are also the most important abnormal signs during the neonatal period

In order to make a more detailed study of the small for gestational-age infants three sub-samples were selected within the investigated material

1 18 apparently healthy SGA infants (below −2 SD) All but one were full term the group is referred to as full term small for gestational age"

? A matched control group of infants with normal birth weights (between −2 and +2 SD) and of the same sexes and gestational ages as the corresponding SGA infants was selected 18 infants in all The maximal difference in gestational age between an infant in this group and the corresponding SGA infant was ? days The mean gestational age was identical in the two groups The control group is referred to as full term appropriate for gestational age"

3 Another matched control group of 18 pre term AGA infants (birth weight between −2 and +2 SD) was selected The maximal difference in birth weight between an infant in this group and the corresponding SGA infant was 40 g The mean weights in the two groups were identical In three cases it was not possible to select a pre term infant of the same sex as the SGA infant (in all three cases a boy had to be selected instead of a girl) This group is referred to as pre term appropriate for gestational age"

Seven infants in the SGA group had crown-heel length below −2 SD whereas all th other infants had normal crown heel length There were no differences between these thre groups regarding age weight, height, parity or smoking habits of the mothers Seven mothers in the SGA group had moderate toxemia during pregnancy as compared with 3 in the pre term group and none in the full term AGA group Six of the mothers in the pre term group had bleedings during pregnancy as compared with 3 in the SGA group and none in the term AGA group

In a corresponding way a group of 9 large for gestational-age infants was selected The upper limit of the 90th percentile was chosen since only 4 infants had a birth weight above +2 SD A control group of infants of the same sexes and gestational ages (birth weights between the 10th and 90th percentiles) was selected The maximal age difference between corresponding infants in the two groups was 5 days The mean gestational age was the same for the two groups.

Table 3 *Important abnormal signs noticed during the perinatal period in the investigated material of 174 infants*

	Number
Low fetal heart rate during delivery	13
Postnatal asphyxia (Apgar score below 7 at 1 or 10 min after birth)	26
IRDS	4
Other respiratory abnormalities	12
Obvious neurological abnormalities	5
Isoimmunization (anti D anti A, and other)	10
Hyperbilirubinaemia (bilirubin above 15 mg per 100 ml)	45
Minor anomalies	5

METHODS

Immediately afer birth a midwife weighed the infant on a balance (Stathmos 397 Lindell AB Jonkoping Sweden) as a part of the routine care The weight was registered and recorded to within 5 g The crown heel length was measured by the same personnel using a measuring board with supports for the head and feet The length was recorded to within 0 5 cm Th nearest length in whole centimetres was used in calculations Head circumference was measured on the 1st or 2nd day by the author using a tape measure The largest occipito-frontal head circumference was recorded to within 1 cm The skull diameters were measured at the same time using a caliper The values were recorded to the nearest whole centimetre

Control of methods

The weighings and measurem nts of birth weight and crown-heel length were controlled as follows Three nurses independently using the same instruments weigh d and measured 19 infants not included in the study They were given no previous instructions in the technique Each infant was weighed by the three nurses within a short period of time thus avoiding natural variations in weight Th maximal difference in the weights obtained by the three investigators was 50 g in one case 35 g in one case in the rest of the cases it was 0–25 g The m an difference was 2 g No systematic differences were seen Th maximal difference in the crown heel lengths obtained by the three investigators was 2.5 cm in one case In the other infants it was 0–1.5 cm The m an difference was 0 2 cm No systematic differences were seen

The measurements of head circumference and skull diameters was controlled in the following way The author made the masurements on 10 infants and the values were recorded A couple of hours later on the same day the measurements were repeated on the same infants, the values from the first examination not being available at the second examination There

Table 1 *Investigated material divided according to sex into gestational age groups of 2 week intervals*

Gestational age (days)	Total material	Boys	Girls
<225	6	6	0
225–238	10	7	3
239–252	18	10	8
253–266	29	15	14
267–280	42	19	23
281–294	51	22	29
>294	18	9	9
Total material	174	88	86

AGA = appropriate for gestational age Infant with birth weight within normal limits for the gestational age (between −2 and +2 S D or between the 10th and 90th percentiles)

LGA = large for gestational age Infant with birth weight above the 90th percentile according to Swedish standard curves

Pre term gestational age less than 267 days postmenstrual

Term gestational age between 267 and 294 days

Post term gestational age more than 294 days postmenstrual

LMP = last menstrual period

Maturity as used by the author maturity is an expression of the degree of development of the newborn infant Maturity thus defined is dependent on the gestational age of the infant but also on other factors such as biological variation (9)

MATERIALS

Criteria for selection

Only infants for whom reliable information about gestational age was available were included in the study The mothers were interviewed by the author on one of the first days after birth All records from the prenatal visits were checked The following criteria had to be fulfilled for inclusion

1 The mother could state the exact date for the beginning of her last menstrual period (LMP) at the interview This date should be the same as that earlier given at the first antenatal visit

2 The menstrual cycles had been regular at intervals of 28 ±3 days before pregnancy

3 The LMP should have been normal with respect to duration and amount and occurred on the expected date

4 If oral contraceptives were used before pregnancy at least one spontaneous menstruation should have occurred before pregnancy

5 There should have been no bleeding during the first 2 months after the LMP

6 The time from the LMP to the recognition of fetal movements should have been within the following limits for primipara fetal movements no earlier than the 18th week and no later than the 22nd for multipara no earlier than the 16th and no later than the 20th week

Investigated material

The investigations were performed during the course of one year from November 1967 to November 1968 During this period about 270 mothers were interviewed The above criteria were fulfilled for 187 infants who comprised the primary material The main reason for excluding the other infants was irregular menstrual cycles The primary material consisted of two parts Part one included all low birth weight infants (≤2 500 g) born during this time at Umeå University Hospital who fulfilled the above mentioned criteria for selection 73 infants in all As the examination of these infants progressed the second part of the material was selected by scanning the hospital records two to three times per week for recently newborn infants with birth weights above 2 500 g The aim was to include as many infants as possible with extreme combinations of birth weight and gestational age who fulfilled the criteria for selection In case no such infant was available a full term normal birth weight infant was studied The second part of the material consisted of 114 infants From the first part of the material studied 13 infants had to be excluded before analysing the results 10 died and 1 was referred to another hospital These infants were incompletely examined Two infants had chromosomal abberrations The remaining material called the *investigated material* thus consisted of 174 infants The material divided according to sex and in gestational age groups of two week intervals is shown in Table 1 In Table 2 the infants are divided according to birth weight in groups of 500 g intervals When the material was divided according to the relation of birth weight to gestational age (Swedish standard curves 6 20) there were 19 infants below −2 S D 145 between −2 and +2 S D and 4 infants above +2 S D 48 infants had birth weights below the 10th percentile 111 between the 10th and 90th percentile and 9 above the 90th percentile Six infants

Table 2 *The investigated material divided according to birth weight into groups of 500 gram intervals*

Birth weight g	Total material
≤1 500	4
1 501–2 000	16
2 001–2 500	40
2 501–3 000	35
3 001–3 500	25
3 501–4 000	32
4 001–4 500	21
>4 500	1

could not be classified according to the standard curves which do not consider gestational ages below 33 weeks (??5 days)

The mean age of the mothers at delivery was 26 years S D 5 3 The youngest mother was 18 years the oldest 42 years 93 were primipara. No mother had had any s rious diseases during pregnancy 17 had had moderate toxemia (blood pressure above 90 mmHg diastolic in combination with albuminuria) 30 mothers had had minor bleeding at some time during pregnancy Seven infants were delivered by ca sarean section the rest by vaginal delivery There were 8 breech d liveries and 2 vacuum extractions The frequencies of low fetal heart rate and low *Apgar scores are seen in Table 3 as are also the most* important abnormal signs during the neonatal period

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Control of methods

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The measurements of head circumference and skull diamet rs was controlled in the following way The author made the masurements on 10 infants and the values were recorded *A couple* of hours later on the same day the *measurements* were repeated on the same infants, the values from the first examination not being available at the second examination. There

Table 4 *Mean values for five anthropometric parameters for infants divided into gestational age groups of 2 week intervals*

The values for head circumference and the two skull diameters are lacking for one infant (278 days) and the two skull diameters for one additional infant (246 days)

	<225	225–238	239–252	253–266	267–280	281–294	295–308
Mean birth weight g							
All infants	1 460	2 048	2 248	2 626	2 954	3 536	3 663
Boys	1 460	2 061	2 235	2 606	3 075	3 730	3 781
Girls	—	2 017	2 265	2 648	2 854	3 390	3 544
Mean crown-heel length cm							
All infants	41 3	45 3	46 1	47 6	48 8	50 6	51 7
Boys	41 3	45 6	46 2	47 3	49 4	51 6	52 7
Girls	—	44 7	46 0	47 8	48 3	49 8	50 7
Mean head circumference cm							
All infants	28 3	30 8	31 8	32 8	33 5	34 8	35 6
Boys	28 3	31 1	32 2	32 9	34 0	35 6	36 1
Girls	—	30 0	31 3	32 6	33 0	34 2	35 0
Mean occipito-frontal diameter cm							
All infants	10 0	10 5	10 8	10 9	11 3	11 7	12 2
Boys	10 0	10 7	11 0	11 1	11 5	12 0	12 4
Girls	—	10 0	10 6	10 8	11 2	11 4	11 9
Mean bi parietal diameter cm							
All infants	7 5	8 0	8 6	8 9	9 3	9 5	10 2
Boys	7 5	8 0	8 8	8 9	9 4	9 6	10 8
Girls	—	8 0	8 3	8 9	9 1	9 4	9 6
Number boys/girls	6/0	7/3	10/8 (7)	15/14	19/23 (22)	22/29	9/9

were no differences in the measurements of head circumference For the occipito frontal diameter there was a difference of 1 cm for one child and for bi parietal diameter there was a difference of 1 cm for three infants

All the methods used for anthropometric measurements thus gave an acceptable degree of reproducibility

Statistical methods

The methods of sampling individuals de cribed above are such that the *characteristics* (parameters) measured can be regarded as random samples

From the statistical standpoint the aim of the present study was to obtain equations *for estimating* the length of gestation (Y) from other characteristics (X_1 X_p) measured In order to obtain these relations between gestational age (Y) as dependent variable and *different combinations of the other characteristics* as independent variables multiple regression analysis was used For further details about model building see e g Draper & Smith (5)

There was reason to believe that *many of the variables* measured would give approximately the same information about gestational age To obtain the best equation for estimating gestational age using a reduced number of variables the data were analysed by a stepwise multiple regression procedure (5) using a computer (CD 3200) and a standard program (BMD O2R version of June 2 1964 Health Sciences Computing Facility UCLA) A number of diff rent models were examined and compared Among these the relationship between the period of gestation and the total maturity score (8) can be mention d

The 95° confidence limits for predicting the unknown value of Y from a new observed value (X) of the independent variables was calculated according to the following formula

$$\hat{Y}_0 \pm t_{0\,975}^{(n-p-1)} \sqrt{MSe}\sqrt{1+X_0'(X'X)^{-1}X_0}$$

wh re $\hat{Y}_0$ is the predicted value of Y X_0 the vector value of th independent variables of the new observation and X is the matrix of the independent variabl s in the original sample (5 14) The multiple correla tion co fficient *was also calculated*

The data for boys and girls could be compared by comparing the separat regression lines with respect to slope and position according to Brownlee (2)

For calculating *the arithmetic mean and standard* deviation

$$\left(S\,D_x = \sqrt{\frac{\Sigma(Y_i-\bar{Y})^2}{n-1}}\right)$$

conventional statistical methods were applied

RESULTS

The *mean values* for the five anthropometric parameters studied, infants divided into gesta

tional age groups of 2 week intervals are seen in Table 4 which also gives the results for boys and girls separately There are minor differences between boys and girls in all the age groups

In Figs 1 and 2 the values for two of the parameters birth weight and head circumference are plotted against gestational age Boys and girls are not separated in these figures There is a wide scatter around the linear regression line (see below) especially for birth weights in the low range The *five parameters were correlated to gestational age* using linear regression analysis The coefficients for the correlations are presented in Table 5 for boys and girls separately as well as for all infants All correlations were significant ($p<0\ 001$) The highest correlation was for head circumference This is true for the group as a whole as well as for boys and girls separately The linear regression equations for Y (gestational age) on X (head circumference) were

$Y=11\ 03+7\ 75\ X$ (all infants)

$Y=-21\ 46+8\ 57\ X$ (boys)

$Y=52\ 54+6\ 65\ X$ (girls)

If the common formula for both sexes is used in calculating gestational age the gestational age of boys is underestimated by about 9 days for low values and 3 days for high values of head circumference compared with the formula for

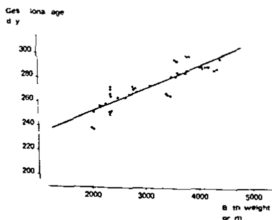


Fig 1 Birth weight plotted against g stational age Linear regression line indicated in the figure $N=174$

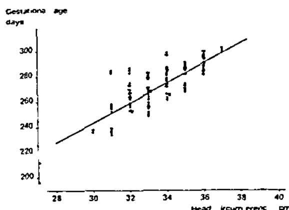


Fig 2 Head circumference plotted against gestational age Linear regression line indicated in the figure $N=173$

boys only The gestational age of girls will be overestimated by about 10 days for low values and about 3 days for high values of head circumference The corresponding equation for birth weight was $Y=214\ 61+0\ 02\ X$ Logarithmic functions did not give a significantly better expression of the relations between the five parameters and gestational age than did the linear regression analyses

The 95% *confidence limits* for estimating gestational age from different values for head circumference (boys and girls together) were $\pm 26\ 5$ days (mean value for infants younger than 225 days) $\pm 26\ 1$ days (mean value for all infants) $\pm 26\ 1$ days (mean value for infants older than 294 days) The confidence limits for birth weight (mean value) were $\pm 28\ 9$ days and for crown–heel length (mean value) $\pm 29\ 7$ days

An attempt was made to analyse the anthropometric parameters together in stepwise multiple regression analysis but only head circumference entered the multiple regression at a significant ($p<0\ 05$) F value Thus the use of a combination of anthropometric measurements did not significantly increase the precision of estimating gestational age Analysing only birth weight and crown–heel length together gave confidence limits of $\pm 28\ 6$ days

The confidence limits for estimating gestational age from mean values of head circumfe

Table 4 *Mean values for five anthropometric parameters for infants divided into gestational age group of 2 week intervals*

The values for head circumference and the two skull diameters are lacking for one infant (278 days) and the tw skull diameters for one additional infant (246 days)

	<225	225–238	239–252	253–266	267–280	281–294	295–308
Mean birth weight g							
All infants	1 460	2 048	2 248	2 626	2 954	3 536	3 663
Boys	1 460	2 061	2 235	2 606	3 075	3 730	3 781
Girls	—	2 017	2 265	2 648	2 854	3 390	3 544
Mean crown-heel length cm							
All infants	41.3	45.3	46.1	47.6	48.8	50.6	51.7
Boys	41.3	45.6	46.2	47.3	49.4	51.6	52.7
Girls	—	44.7	46.0	47.8	48.3	49.8	50.7
Mean head circumference cm							
All infants	28.3	30.8	31.8	32.8	33.5	34.8	35.6
Boys	28.3	31.1	32.2	32.9	34.0	35.6	36.1
Girls	—	30.0	31.3	32.6	33.0	34.2	35.0
Mean occipito-frontal diameter cm							
All infants	10.0	10.5	10.8	10.9	11.3	11.7	12.2
Boys	10.0	10.7	11.0	11.1	11.5	12.0	12.4
Girls	—	10.0	10.6	10.8	11.2	11.4	11.9
Mean bi parietal diameter cm							
All infants	7.5	8.0	8.6	8.9	9.3	9.5	10.2
Boys	7.5	8.0	8.8	8.9	9.4	9.6	10.8
Girls	—	8.0	8.3	8.9	9.1	9.4	9.6
Number boys/girls	6/0	7/3	10/8 (7)	15/14	19/23 (22)	22/29	9/9

were no differences in the measurements of head circumference. For the occipito frontal diameter there was a difference of 1 cm for one child and for bi parietal diameter there was a difference of 1 cm for three infants

All the methods used for anthropometric measurements thus gave an acceptable degree of reproducibility

Statistical methods

The methods of sampling individuals described above are such that the *characteristics* (parameters) measured can be regarded as random samples

From the statistical standpoint the aim of the present study was to obtain equations for estimating the length of gestation (Y) from other characteristics (X_1 ... X_p) measured. In order to obtain these relations between gestational age (Y) as dependent variable and different combinations of the other characteristics as independent variables multiple regression analysis was used. For further details about model building see e g Draper & Smith (5)

There was reason to believe that many of the variables measured would give *approximately the same* information about gestational age. To obtain the best equation for estimating gestational age using a reduced number of variables the data were analysed by a stepwise multiple regression procedure (5) using a computer (CD 3200) and a standard program (BMD O2R *version of June 2 1964* Health Sciences Computing Facility UCLA) A number of different model were examined and compared. Among these th relationship between the period of gestation and th total maturity score (8) can be mention d

The 95 % *confidence limits for predicting the un known value of Y from a new observed value* (X_0) o the independent variables was calculated accordin *to the following formula*

$$\hat{Y}_0 \pm t_{0.975}^{(n-p)} \sqrt{MSe\,(1 + X_0'(X'X)^{-1} X_0)}$$

where $\hat{Y}_0$ is the predicted value of Y X_0 the vecto value of the independent variables of the new observa tion and X is the matrix of the independent variabl s in the original sample (5 14) The multiple correla tion co fficient was also calculated

The data for boys and girls could be compared by comparing the separat regression lines with re spect to slope and position according to Brownlee (2)

For calculating the arithmetic mean and standard deviation

$$\left(S\,D_x = \sqrt{\frac{\Sigma(X_i - \bar{X})^2}{n-1}}\right)$$

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RESULTS

The *mean values* for the five anthropometric parameters studied infants divided into gesta

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In Figs 1 and 2 the values for two of the parameters birth weight and head circumference are plotted against gestational age Boys and girls are not separated in these figures There is a wide scatter around the linear regression line (see below) especially for birth weights in the low range The *five parameters were correlated to gestational age* using linear regression analysis The coefficients for the correlations are presented in Table 5 for boys and girls separately as well as for all infants All correlations were significant ($p < 0\ 001$) The highest correlation was for head circumference This is true for the group as a whole as well as for boys and girls separately The linear regression equations for Y (gestational age) on X (head circumference) were

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If the common formula for both sexes is used in calculating gestational age the gestational age of boys is underestimated by about 9 days for low values and 3 days for high values of head circumference compared with the formula for

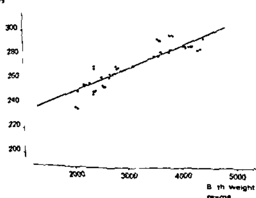


Fig 1 Birth weight plotted against gestational age Linear regression line indicated in the figure $N = 174$

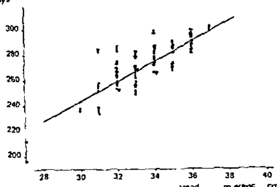


Fig 2 Head circumference plotted against gestational age Linear regression line indicated in the figure $N = 173$

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The confidence limits for estimating gestational age from mean values of head circumfe-

Table 5 *Coefficients for the correlation between gestational age and five anthropometric measurements*

	Birth weight (g)	Crown–heel length (cm)	Head circum ference (cm)	Occipito–frontal diameter (cm)	Bi parietal diameter (cm)
All infants	0 72	0 71	0 78	0 65	0 56
Boys	0 80	0 78	0 87	—	—
Girls	0 62	0 60	0 70	—	—

Table 6 *Anthropometric data, comparison between small for gestational age, pre term and full term appropriate for gestational age infants*

Asterisks indicate significant differences between means

	Number	Age at birth (days)	Birth weight (g)	Crown–heel length (cm)	Head circum ference (cm)	Occipito–frontal diameter (cm)	Bi parietal diameter (cm)
Full term SGA infants	18	276	2 133	46 0	31 9	10 8	8 8
			***	***	***	**	***
Full term AGA infants	18	276	3 399	49 7	34 2	11 4	9 5
		***	***	***	***	***	***
Pre term AGA infants	18	242	2 134	45 5	31 2	10 7	8 3

(Bracket on Bi parietal diameter between Full term SGA and Pre term AGA infants: *)

* $p<0\ 05$ ** $p<0\ 01$ *** $p<0\ 001$

rence for boys and girls separately were ±23 9 days and ±24 5 days respectively

The mean values for the anthropometric data in 18 *small for-gestational-age infants* (as defined above) as compared with pre term and term AGA infants are shown in Table 6 Not only birth weight but also the other four parameters were significantly lower in SGA than in AGA infants No significant differences were found between SGA infants and pre term AGA infants with respect to birth weight (see principles for the selection of these two groups) but this was true also for crown–heel length, head circumference and occipito frontal diameter Only the bi parietal diameter was significantly lower in the pre term group

The corresponding values for nine *large for gestational age infants* (as defined above) and their control infants of normal birth weight is shown in Table 7

Table 7 *Anthropometric data comparison between mean values for large for gestational age infants and appropriate for gestational age infants*

Asterisks indicate significant differences

	Number	Age at birth (days)	Birth weight (g)	Crown–heel length (cm)	Head circum ference (cm)	Occipito–frontal diameter (cm)	Bi parietal diameter (cm)
LGA infants	9	287	4 358	52 7	35 6	11 8	9 8
			***	**			
AGA infants	9	285	3 307	50 2	34 6	11 6	9 7

** $p<0\ 01$ *** $p<0\ 001$

DISCUSSION

I *Estimation of gestational age from LMP*

In all studies on maturity in fetuses and newborn infants a crucial point is the calculation of the gestational age from the mother s information as to the LMP since the exact date of conception is rarely known The LMP must be carefully controlled by interviewing the mother and checking the dates given at prenatal visits The menstrual cycle must have been regular and there should have been no bleeding during early pregnancy which might have been erroneously taken for a menstruation Ideally an early vaginal examination should be performed in order to estimate the uterine size However this had been done in only about half of the mothers in the present study Different values are given in the literature for the time interval between the LMP and quickening Nonetheless recording the time when fetal movements are first noted increases the accuracy of estimation of the gestational age as recently pointed out by Rawlings & Moore (19) The time interval chosen for this study 20 ± 2 weeks for primipara 18 ± 2 weeks for multipara agrees well with the mean and standard deviation given by Rawlings & Moore The use of oral contraceptives can postpone the time for ovulation after cessation of medication Of 14 mothers of infants in this study who had used oral contraceptives 4 had had one spontaneous menstruation before conception the others had had 2 or more

There is thus reason to believe that the gestational ages calculated from the information supplied by the mothers were correct and that the variations in results to be discussed below do not depend on errors in calculating gestational age from the LMP It should be pointed out however that the interval between LMP and ovulation varies (9)

II *Definitions*

The definitions used have been arbitrarily chosen As to the definition of pre and post term delivery, an international recommendation from 1970 is to place the limit for pre term 3 weeks before term and for post term 2 weeks after term (7) It was found convenient however to divide the infants in the present study into gestational age groups of 2 week intervals thus defining pre term as 2 weeks before term This is also in accord with suggestions of Battaglia et al (1) and others

The definition of weeks used (i e 40th week = days 274–280) is in agreement with the definitions used in constructing the Swedish standard curves (6 20) but differs from recent international recommendations (40th week = days 280–286 (7))

SGA was defined here as birth weight below −2 S D The two most used definitions are below −2 S D (roughly corresponding to the 3rd percentile) and below the 10th percentile There were two main reasons for choosing the −2 S D limit. First it was considered more interesting to study a group with relatively pronounced growth retardation Secondly it was possible within the investigated material to select matched control groups to the infants below −2 S D This would not have been possible had the 10th percentile limit which selects many more infants been chosen

III *Results*

The present material of 174 infants was selected to contain a high proportion of pre term and SGA infants The latter fact is reflected in the lower mean weights for different gestational age groups as compared with those given in the Swedish standard curves (6 20)

In correlating the five anthropometric parameters studied to gestational age at birth (linear regression analyses) it was found that crown-heel length did not correlate any better than birth weight to gestational age whereas head circumference had a higher degree of correlation This is important to note since head circumference is a simple but neglected anthropometric measurement there are few standard curves relating it to gestational age The high correlation coefficients for head circumference as compared with the other parameters was

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** $p<0\ 01$ *** $p<0\ 001$

is proportionally higher than the weights of most other organs (10, 17) Brain weight and head circumference are however sometimes disproportionate (17)

Parmelee et al (18) found that less than half of their infants (all low birth weight infants) with birth weights below the 10th percentile had head circumferences between the 10th and 90th percentiles It is not stated how many of the infants with appropriate birth weight had head circumferences below the 10th percentile The limit of -2 S D as chosen in the present study reflects a much more pronounced retardation of growth than does the 10th percentile It is important not to draw general conclusions from comparisons between samples of SGA infants if they are not selected in comparable ways Comparable selection means that the limits chosen (percentiles or S D) should be the same and that the standard curves used should be appropriate for the sample i e constructed for the type of population from which the sample is selected The widespread use of the Denver curves (15 16) is a source of error in this context since they were constructed from a population living at high altitude

The *large for gestational-age* infants in the present material did not differ significantly from their AGA controls with respect to head circumference or the two skull diameters However the samples were very small and thus no definite conclusions can be drawn from the results

SUMMARY

Five anthropometric measurements birth weight crown-heel length head circumference occipito-frontal diameter and bi parietal diameter were recorded in a group of 174 newborn infants of various gestational ages

The material was selected to contain a relatively large number of infants with extreme birth weights and/or gestational ages

Head circumference was better correlated to gestational age than were the other four measurements Birth weight and crown heel length had the same degree of correlation to gestational age The two skull diameters showed significantly lower correlations to gestational age The confidence limits for estimating gestational age on the basis of the mean value for head circumference were ± 26.1 days in this material

In SGA infants crown-heel length and head circumference were not significantly greater than in pre term infants of the same birth weight

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evident for boys and girls separately as well as for the group of infants as a whole The differences in coefficients between boys and girls probably depend mainly on differences in age distribution (9) There are more boys among the pre-term infants, which is reflected in a greater standard deviation for age 24 0 days as compared with 16 9 for girls

The occipito-frontal diameter and the biparietal diameter were considerably less well correlated to gestational age than was birth weight This finding obviously casts some doubt on the value of single measurements of bi parietal diameter in prenatal assessment of maturity, regardless of the method used, radiological examination (13) or ultrasonics (4, 3)

As can be expected from the correlation coefficients, the 95% *confidence limits* for estimating gestational age from anthropometric measurements were broader for birth weight than for head circumference If anthropometric measurements are to be used in maturity assessment head circumference is to be preferred to birth weight crown-heel length or skull diameters The estimation of age from head circumference alone is better than from radio logical measurements of epiphyseal centres or from measuring motor conduction velocity but not as good as from evaluating external characteristics or neurological tests (9) Although the confidence limits presented here are fairly broad largely depending on the age distribution within the sample, it can be concluded that measuring head circumference is of some practical importance in assessing maturity especially if combined with other methods for maturity assessment (9)

The use of a combination of the anthropometric parameters studied did not significantly increase the precision of estimating gestational age, since only head circumference entered the stepwise multiple regression at significant F-value This is in accord with the findings of Parmelee et al (18) in premature infants Timonen et al (22) on the other hand, in a large Finnish material found that birth weight and crown heel length together estimated gestational age as well as did these measurements combined with head circumference They did not state how head circumference was measured In Scandinavia the midwives usually measure head circumference from the lowest point of the occiput to the forehead, in which case the largest head circumference is not measured In the present material, head circumference was always given as the largest measurement

It is well known that crown-heel length may be reduced in small for-gestational age infants, although not necessarily to the same extent as birth weight (10) According to Usher et al (23) the head circumference is also reduced These authors conclude that body proportions are similar in SGA infants and pre term infants of the same birth weight and are therefore of no value as indices of maturity In this series there were no significant differences in crown-heel length, head circumference or occipito frontal skull diameter between SGA and pre term AGA infants of the same birth weight So although head circumference is a much better index of maturity than birth weight or crown-heel length its value in differentiating pre term AGA and SGA infants of the same weights is limited The definition used for SGA infants (below −2 S D) means however that only infants of markedly low birth weight were included The SGA group in the present material was not homogeneous Only 7 were short for gestational age as well Excluding these 7 infants together with their respective control infants did not however significantly change the result of the comparison between the three groups

The bi parietal diameter on the other hand, was significantly lower in pre-term AGA infants than in SGA infants This probably reflects the well known fact that pre term infants often have a rather flattened head (from side to side) This difference is of limited practical use however since the range in bi parietal diameter is wide even at a constant gestational age The finding of a smaller head circumference in SGA infants seems to be inconsistent with the fact that brain weight in these infants

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CONTROL OF SODIUM HOMEOSTASIS IN CHILDREN WITH RECURRENT URINARY TRACT INFECTIONS AND REDUCED GLOMERULAR FILTRATION RATES

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Sodium is the dominant cation in the extracellular fluid including plasma It is thus the dominant cation in the filtrate passing the glomerular membrane In renal insufficiency phosphate and potassium retention secondary to reduced filtration rates are common Yet sodium retention is rarely encountered during renal insufficiency This suggests an unusual control of sodium homeostasis even in advanced renal disease Studies by Slatopolsky et al have provided much information on sodium homeostasis in adult patients with renal insufficiency of various etiologies (16) In a previous series of studies we have attempted to clarify the pathophysiology of recurrent urinary tract infections in childhood (1 3) In patients with changes in the renal parenchyma demonstrated by intravenous pyelograms a wide range of functional disturbances were observed The dominant finding was a more or less pronounced reduction of the filtration rate This report deals with the control of sodium homeostasis in the children with recurrent urinary tract infections and a wide range of filtration rates but without clinical signs of renal insufficiency The material studied was selected to obtain the greatest possible homogeneity in the pathophysiology Thus only patients with nonacute recurrent urinary tract infections uncomplicated by other renal diseases were studied It was also thought that overlooked diseases in circulatory and hepatic organs that might influence sodium homeostasis are much more rare in children than in adults Only children older than 6 years were studied At that age the kidneys can be expected to work with full capacity

The study was designed to provide information on the long time adaptation to renal disease as well as the ability to adapt to a short time oral or intravenous sodium load Since the physiological control of sodium reabsorption and in a broader sense of sodium homeostasis and extracellular fluid volume is only partly known (4 5 19 20) the interpretation of the results will have to be speculative

Supported by the Swedish Medical Research Council Grant B67 19X 2049-01A.

MATERIAL AND METHODS

Material

17 patients aged 6 to 17 years were studied All the patients had previous histories of urinary tract infections confirmed by urine cultures No patient had any sign of infection during the study or at least two months prior to the study In only two of the patients the blood urea nitrogen concentration exceeded 22 mg per 100 ml (40 and 31 mg per 100 ml respectively) Three of the patients had moderate elevation of the arterial blood pressure An intravenous pyelo-

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Sodium is the dominant cation in the extracellular fluid including plasma It is thus the dominant cation in the filtrate passing the glomerular membrane In renal insufficiency phosphate and potassium retention secondary to reduced filtration rates are common Yet sodium retention is rarely encountered during renal insufficiency This suggests an unusual control of sodium homeostasis even in advanced renal disease Studies by Slatopolsky et al have provided much information on sodium homeostasis in adult patients with renal insufficiency of various etiologies (16) In a previous series of studies we have attempted to clarify the pathophysiology of recurrent urinary tract infections in childhood (1 3) In patients with changes in the renal parenchyma demonstrated by intravenous pyelograms a wide range of functional disturbances were observed The dominant finding was a more or less pronounced reduction of the filtration rate This report deals with the control of sodium homeostasis in the children with recurrent urinary tract infections and a wide range of filtration rates but without clinical signs of renal insufficiency The material studied was selected to obtain the greatest possible homogeneity in the pathophysiology Thus only patients with nonacute recurrent urinary tract infections uncomplicated by other renal diseases were studied It was also thought that overlooked diseases in circulatory and hepatic organs that might influence sodium homeostasis are much more rare in children than in adults Only children older than 6 years were studied At that age the kidneys can be expected to work with full capacity

The study was designed to provide information on the long time adaptation to renal disease as well as the ability to adapt to a short time oral or intravenous sodium load Since the physiological control of sodium reabsorption and in a broader sense of sodium homeostasis and extracellular fluid volume is only partly known (4 5 19 20) the interpretation of the results will have to be speculative

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MATERIAL AND METHODS

Material

17 patients aged 6 to 17 years were studied All the patients had previous histories of urinary tract infections confirmed by urine cultures No patient had any sign of infection during the study or at least two *months* prior to the study In only two of the patients the blood urea nitrogen concentration exceeded 22 mg per 100 ml (40 and 31 mg per 100 ml respectively) Three of the patients had moderate elevation of the arterial blood pressure An intravenous pyelo-

Table 1 *Arterial blood pressure, glomerular filtration rate during water diuresis and IVP findings in patients subjected to intravenous saline load*

Patients	Blood pressure	GFR (ml/min/1 73 m² b s)	Left renal parenchyma	Right renal parenchyma
SN	130/90	28	Small in size Uneven in shape	Small in size Uneven in shape
KH	110/70	32	Small in size Uneven in shape	Small in size Uneven in shape
IH	105/80	32	Small in size Uneven in shape	Small in size Uneven in shape
ILL	110/70	39	Small in size Even in shape	Normal in size Uneven in shape
LN	130/100	51	Small in size Uneven in shape	Moderately reduced in size Uneven in shape
IBS	125/90	82	Small in size Uneven in shape	Moderately reduced in size Uneven in shape
BS	110/70	96	Normal in size Even in shape	Small in size Uneven in shape
EJ	105/55	98	Normal in size Uneven in shape	Normal in size Uneven in shape
KO	100/60	104	Normal in size Even in shape	Normal in size Even in shape
IO	110/70	110	Normal in size Even in shape	Normal in size Even in shape
AE	110/65	112	Moderately reduced in size Uneven in shape	Small in size Uneven in shape
SA	110/55	122	Normal in size Uneven in shape	Normal in size Uneven in shape

gram (IVP) had been carried out in all the patients within a period of at most two years A tabular summary of the blood pressures glomerular filtration rates during water diuresis and the radiological findings of the patients submitted to intravenous saline loading are given in Table 1

The studies were performed while the patients were in the paediatric metabolic ward S t Goran s Hospital All the patients were kept on a constant sodium intake 75–100 mEq/1 73 m² body surface daily 3 to 4 days before each test or hormone determination was carried out

Studies of basal sodium excretion

The determination of the 24 hour urinary sodium excretion was generally carried out on the 3rd day of controlled diet

Studies on the elimination of an oral sodium load

All the patients reported were submitted to this study In order to ensure a high and constant urine flow the patients were allowed to drink water in an amount corresponding to 2–2 5% of the body weight initially and thereafter amounts slightly exceeding the diuresis every 30 min Urine was collected by spontaneous voiding at hourly intervals After collection of one 60 min control urine sample sodium chloride was given by mouth in a dosage of 95 mEq sodium/1 73 m² body surface Following the administration of NaCl 5–6 urine samples were collected Since none of the patients suffered from diarrhoea or other gastrointestinal disorders the fecal excretion was assumed to be a constant and a rather insignificant factor in the elimination of the extra sodium load

Clearance studies during water diuresis with superimposed hypotonic saline load

All the patients reported were submitted to clearance studies during water diuresis, In 12 of the patients the study was completed with a hypotonic saline load

Water diuresis was induced in the same way as described under the oral test When a constant diuresis had been obtained within about 60 to 90 min 3 5 clearance periods were performed In 12 patients the clearance studies were then continued during the infusion of hypotonic saline 0 5 % and 0 7 % at a rate slightly exceeding the diuresis The general proceeding for the latter part of the study was 3–5 10 min clearance periods during the infusion of 0 5% NaCl and 3–5 clearance periods during the infusion of 0 7% NaCl The hypotonic saline was infused into an inferior vena cava catheter introduced via the femoral vein by Seldinger technique In a few cases the studies were discontinued when the patients started to feel any discomfort such as nausea or chill The last period was then not counted In all the patients however the study could be continued until the saline infusion had resulted in an accumulation of 70 mEq Na^+/1 73 m² b s This was generally obtained during the first periods of the infusion of 0 7 % NaCl

Standard clearance techniques were used The glomerular filtration rate was determined by the clearance of inulin (Laevasar Gesellschaft) For urine sampling a double lumen polyethylene catheter was used enabling continuous suction Blood samples were taken in the middle of each urine sampling period from an indwelling catheter in a peripheral vein

Clearance studies during the transition from hydropenia to water diuresis

Those studies were all performed 1 2 2 years prior to the salt balance studies Nine of seventeen patients reported were subjected to this study The protocol for these studies have been reported earlier (1) Three of the present nine patients were included in the earlier presented material

Hormone analyses

On the fourth day of controlled diet the 24 hour

Table 2 *Urinary sodium excretion (μEq/min/1 73 m² b.s) in patients with low and high GFRs*

	Urinary sodium excretion (μEq/min/1 73 m² b s)			
	24 hour	Hydropenia	Water diuresis	Hydropenia versus water diuresis
Patients with low GFRs	100±26	135±34	205±48	$0 02 < p < 0 05$
n	5	4	5	
Patients with high GFRs	110±37	128±36	237±34	$p < 0 01$
n	6	5	7	
Patients with low GFRs versus patients with high GFRs	$0 6 < p < 0 7$	$0 8 < p < 0 9$	$0 2 < p < 0 3$	

excretion of aldosterone 17-oxosteroids and 17 hydroxycorticosteroids were measured Aldosterone was determined by a modification of a double isotope method described by Kliman & Peterson (9) 17-oxo steroids and 17 hydroxycorticosteroids were determined spectrographically (2)

Chemical analyses

Sodium and potassium concentrations in serum and urine were determined by a flame photometer osmolality cryoscopically with a Knauer microosmometer and inulin according to the method of Heyrovsky (7)

For the statistical calculation Student s *t* test has been used

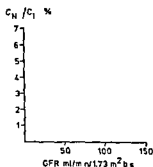


Fig 1 The relationship between glomerular filtration rate (ml/min/1 73 m b s) and fractional sodium excretion (C /C) The values were obtained during water diuresis

RESULTS

Glomerular filtration rate

D pending upon the values for the glomerular filtration rates (GFRs) the patients could b divided into two groups (*a*) patients with severely to mod rately reduced (*low*) filtration rates and (*b*) patients with subnormal to normal (*high*) filtration rates During water diuresis the filtration rates were 36 4±9 1 ml/min/1 73 m b s and 103 4±12 9 ml/min/1 73 m b s for the two groups respectively Mean values ± one standard deviation are given

Basal sodium excretion

The total urinary sodium excretion for the patients with low GFRs and the patients with high GFRs are given in Table 2 The values are obtained from 24 hour urine specimens from clearance studies during hydropenia and from clearance studies during water diuresis The 24 hour urinary sodium excretion averaged 100 μEq/min/1 73 m b s in the patients with low GFRs and 110 μEq/min/1 73 m b s in the patients with high GFRs The difference between the two groups was not significant The urinary sodium excretion during hydropenia was 135 μEq/min/1 73 m b s in the patients with low GFRs and 128 μEq/min/1 73 m b s in the patients with high GFRs The difference between the two groups was not signifi

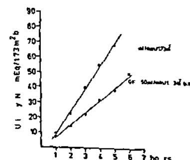


Fig 2 The cumulative sodium excretion each hour following oral sodium load in a patient with glomerular filtration rate of 112 ml/min/1 73 m b s and in a patient with glomerular filtration rate of 50 ml/min/1 73 m b s

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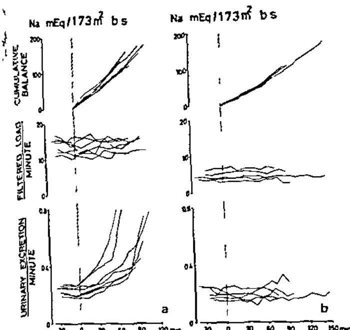


Fig 4 The effect of hypotonic saline infusion on cumulative sodium balance, filtered load of sodium and urinary sodium excretion in patients with high GFRs (*a*) and in patients with low GFRs (*b*) The values to the left of the dashed line are obtained during water diuresis alone The dashed line shows the time when the hypotonic saline load was started (0 min)

saline load in patients with low GFRs In contrast in patients with high GFRs hypotonic saline load resulted in a significant depression in the amount of reabsorbed sodium and thus a significant increase in fractional sodium excretion

Renal response to transition from hydropenia to water diuresis

Table 4 demonstrates the values for glomerular filtration rates filtered sodium reabsorbed sodium urinary sodium excretion and fractional

Table 3 *Changes in glomerular filtration rate filtered reabsorbed and excreted sodium when sodium balance is increased 70 mEq/1 73 m² b.s during water diuresis*

	GFR (ml/min/ 1 73 m b s)	Filtered Na (μEq/min/ 1 73 m² b s)	Reabsorbed Na⁺ (μEq/min/ 1 73 m b s)	U_{Na} V (μEq/min/ 1 73 m b s)	C_{Na}/C_{In} ( )
Patients with low GFRs					
n	5	5	5	5	5
Mean value during water diuresis ± one standard deviation	36 4±9 1	4 849±1 214	4 644±1 220	205±48	4 46±1 64
Difference of mean water diuresis versus accumulated balance of + 70 mEq Na /1 73 m b s	0 4 0 3<*p*<0 4	−5 *p*>0 9	−2 *p*>0 9	−3 *p*>0 9	−0 11 *p*>0 9
Patients with high GFRs					
n	7	7	7	7	7
Mean value during water diuresis ± one standard deviation	103 4±12.9	13 600±1 909	13 362±1 933	237±34	1 82±0 50
Difference of mean water diuresis versus accumulated balance of + 70 mEq Na /1 73 m² b s	−0 3 0 7<*p*<0 8	−81 0 3<*p*<0 4	−260 0 02<*p*<0 05	181 *p*=0 05	1 45 0 02<*p*<0 05

cant The difference in urinary sodium excretion between the 24 hour sampling and the hydropenic sampling is not significant During water diuresis there is a significant increase in urinary sodium excretion in both groups The rise in urinary sodium excretion was somewhat higher in the patients with high GFRs but the difference between the two groups during water diuresis was not statistically significant Thus the excreted amount of sodium found in 24 hour urine specimen as well as during sampling in hydropenia and water diuresis was apparently independent of the glomerular filtration rate To effect this the fraction of filtered sodium excreted must rise when the filtration rate is reduced This is illustrated in Fig 1 where the fractional sodium excretion during water diuresis has been related to the glomerular filtration rate

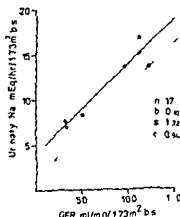


Fig 3 The relationship between the glomerular filtration rate (ml/min/1 73 m b s) and the urinary *elimination rate* (mEq/hr/1 73 m² b s) of an orally administered sodium load All patients studied are included The values for glomerular filtration rate were obtained during water diuresis The urinary sodium elimination rate has been calculated by plotting cumulative sodium excretion against time (Fig 2) *n* Number of patients *b* regression coefficient *s* standard deviation *r* correlation coefficient The solid line represents the line of regression the dashed lines ±2 standard deviations

Renal response to oral sodium load

Fig 2 demonstrates the rate of urinary elimination of an oral sodium load in a patient with high GFR and in a patient with low GFR The accumulated amount of sodium that has been excreted from the time when the last tablet was given has been plotted against time A more rapid sodium elimination in the patient with high GFR was a characteristic finding Fig 3 demonstrates the highly significant ($p<0.01$) correlation coefficient (0 94) between the slope of the sodium excretion line and the filtration rate

Renal response to superimposed hypotonic saline load during water diuresis

Fig 4 *a* and *b* demonstrate the rate of sodium accumulation, the filtered load of sodium and the total urinary sodium excretion in all patients during hypotonic saline loading The patients with high GFRs are represented in Fig 4 *a* and the patients with low GFRs in Fig 4 *b* The filtered load of sodium fluctuated somewhat probably as a consequence of noncharacteristic changes in the GFR In the patients with high GFRs the urinary sodium excretion started to increase continuously already when the accumulated changes in sodium balance exceeded 30 mEq/1 73 m b s When the accumulated changes exceeded 70 mEq/1 73 m b s the rise in urinary sodium excretion became even steeper In contrast the patients with low GFRs demonstrated no characteristic change in urinary sodium excretion when the sodium balance was progressively increased to 70–100–180 mEq/1 73 m b s

Table 3 demonstrates a statistically analysed summary of the effects of the intravenous hypotonic saline load on the renal handling of sodium in all the patients studied The values obtained in water diuresis alone have been compared to those obtained when a positive sodium balance of 70 mEq/1 73 m b s was achieved No significant change in the glomerular filtration rate was seen in either of the two groups As a consequence of the relatively stable GFR the filtered sodium load did not change significantly in either group following the superimposition of the saline load In patients with low GFRs the amount of reabsorbed sodium did not change Thus the fractional sodium excretion was not affected by hypotonic

Table 5 *24 hr urinary excretion of aldosterone 17 oxosteroids and 17 hydroxysteroids in 10 of the patients subjected to intravenous saline loading*

Patients	Aldosterone (μg/24 hr)	17 oxo-steroids (mg/24 hr)	17 hydroxy corticosteroids (mg/24 hr)	
SN male 11 1/12 years	21 4	1 5	2 0	
KH female 16 /12 years	24 8	16 8	21 5	
IH female 17 9/ 2 years	18 6	14 1	20 5	
ILL, female 12 /12 years	12.8	5 5	18 3	
LN female 11 4/12 years	10 0	5 9	11 8	
IBS female 9 9/12 years	6 4	2 0	5 7	
BS female 9 years	19 2	3 6	16 6	
IO female 11 1 / 2 years	11 8	2.3	11 3	
AE, female 14 /12 years	12 5	5 9	15 3	
SA female 9 /12 years	5 4	2 2	3 9	
	9–12 years	12–14 years	14–15 years	15–17 years
Aldosterone normal values μg/24 hr	5	5	5	5
17-oxosteroids normal values				
mg/24 hr mean	1 5	2 5	4 5	7.5
range	0 2–3 0	0 8–5 0	1 8–7 8	3 5–11 0
17 hydroxycorticosteroids normal values				
mg/ 4 hr mean	3 8	5 0	5 7	8 0
range	1 6–7 4	2 0–8 6	3 5–11 0	5 0–12.0

not been calculated before The intravenous saline load in the previous reports were apparently started at a lower level of extracellular fluid volume and the effect i e the inhibition of sodium reabsorption might have been the same effect that was observed during the transition from hydropenia to water diuresis in the present study

There are several possible explanations for the *basal reset of glomerular tubular balance* and thus increased fractional sodium excretion in the patients with low GFRs Since none of the patients were uremic an osmotic diuresis due to retention of poorly reabsorbable solutes can be ruled out

The finding of normal or increased aldosterone excretion and normal 17-oxosteroid and 17 hydroxycorticosteroid excretions practically excluded a decreased stimulation of those hormones

A damage of the renal parenchyma might explain the increased fractional sodium excretion by at least two possible mechanisms (*a*) disease of the renal tubule could result in a depression of the active reabsorptive capacity for sodium Experimental studies have however demonstrated that renal disease is not a prerequisite for the reset of glomerular tubular balance for sodium Thus reset of glomerular tubular sodium balance is also observed in the intact remaining nephrons when the rest of the renal parenchyma is out of function by unilateral nephrectomy and contralateral renal infarction (15) (*b*) If the nephron population is reduced and the remaining nephrons are hyper perfused an increase in fractional sodium excretion *might also occur* This assumes that the amount of sodium and water perfusing individual remaining nephrons will exceed the reabsorptive capacity for sodium Evidence against this hypothesis are presented by the results of the studies during transition from hydropenia to water diuresis The patients with low filtration rates were then able to increase the reabsorptive capacity for sodium despite simultaneous increases in filtration rate i e nephron perfusion rate

Another and perhaps more attractive explanation is that the reset in glomerular tubular balance during low filtration rate is a product of the physiological control mechanisms governing sodium reabsorption. The importance of

Table 4 *Changes in glomerular filtration rate filtered, reabsorbed and excreted sodium during the transition from hydropenia to water diuresis*

	GFR (ml/min/ 1 73 m² b s)	Filtered Na⁺ (μEq/min/ 1 73 m² b s)	Reabsorbed Na⁺ (μEq/min/ 1 73 m² b s)	$U_{Na}V$ (μEq/min/ 1 73 m² b s)	C_{Na}/C_{In} ( )
Patients with low GFRs					
n	4	4	4	4	4
Mean value during hydropenia ± one standard deviation	34 0±15 6	4 794±2 142	4 660±2 115	135±34	3 04±0 61
Difference of mean hydropenia versus water diuresis	13 3	1 606	1 477	129	1 15
	$p<0\ 01$	$p<0\ 01$	$0\ 01<p<0\ 02$	$0\ 01<p<0\ 02$	$0\ 05<p<0\ 1$
Patients with high GFRs					
n	5	5	5	5	5
Mean value during hydropenia ± one standard deviation	95 0±11 1	12 992±1 517	12 864±1 490	128±36	0 98±0 19
Difference of mean hydropenia versus water diuresis	13 7	1 633	1 523	110	0 64
	$0\ 02<p<0\ 05$	$0\ 05<p<0\ 1$	$0\ 05<p<0\ 1$	$p<0\ 01$	$0\ 01<p<0\ 02$

sodium excretion during transition from hydropenia to water diuresis in the patients with low GFRs and in the patients with high GFRs A significant rise in glomerular filtration rate during the transition from hydropenia to water diuresis was found in both groups As a consequence of this there is also a rise in the amount of filtered sodium which is significant in the patients with low GFRs and borderline significant in the patients with high GFRs The amount of reabsorbed sodium is significantly increased in the patients with low GFRs and borderline significantly increased in the patients with high GFRs The urinary sodium excretion was significantly increased in both groups The fractional sodium excretion increased with borderline significance in the patients with low GFRs and with full significance in the patients with high GFRs

Urinary steroid excretion

The 24 hour excretion of aldosterone 17-oxosteroids and 17 hydroxycorticosteroids in 10 of the patients subjected to intravenous saline loading are given in Table 5 The excretion of aldosterone was increased or on the upper limit of the normal values for age given by Visser (18) The excretion of 17 oxosteroids and 17-hydroxycorticosteroids were normal or slightly increased as compared to previous reported values for healthy children in the same age group (10)

DISCUSSION

The control of sodium homeostasis during low glomerular filtration rate has previously been studied during conditions of experimental uremia in dogs where unilateral nephrectomy and 85% infarction of the contralateral kidney had been carried out (15) It has also been studied in a group of patients with uremia of various etiologies including pyelonephritis glomerular nephritis and polycystic kidneys (16) The present observation of an increased fractional sodium excretion and thus a basal reset of glomerular tubular balance for sodium at low levels of GFR is in accordance with those previous studies The present finding of a reduced ability to rapidly adjust to changes in sodium balance in patients with low GFRs is however somewhat in contrast to what has been reported previously This apparent difference might however only be due to variations in experimental design Thus when testing the effect of oral sodium load the elimination rate of sodium has

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ILL, female 17 /$_{12}$ years	12 8	5 5	18 3	
LN female 11 /$_{12}$ years	10 0	5 9	11 8	
IBS female 9^{1} /$_{12}$ years	6 4	2 0	5 7	
BS female 9 years	19 2	3 6	16 6	
IO female 11^{1} /$_{12}$ years	11 8	2 3	11 3	
AE, female 14 /$_{12}$ years	12 5	5 9	15 3	
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Another and perhaps more attractive explanation is that the reset in glomerular tubular balance during low filtration rate is a *product* of the physiological control mechanisms governing sodium reabsorption The importance of

physical forces in the control of sodium reabsorption is well established (11, 17) Thus changes in peritubular capillary hydrostatic and oncotic pressure and in interstitial volume will immediately effect sodium reabsorption No measurement of extracellular fluid volume or plasma volume was made in the present material but there were no clinical signs of fluid retention in the patients with low filtration rates Two of five patients with low GFRs (SN and LN) had only moderate elevations of blood pressure The remaining three patients with low GFRs had normal arterial blood pressure None had hypoproteinemia Thus physical factors do not appear to be of primary importance in the basal reset of glomerular tubular balance

Evidence has also been presented for the existence of a natriuretic factor that would adjust to changes in sodium and fluid homeostasis by affecting the tubular sodium reabsorption (4–6, 13, 14) Although definite evidence for the existence of this additional factor is still lacking, the presence of several factors regulating sodium and fluid homeostasis does not seem unlikely One of the important obstacles to the natriuretic factor theory is the lack of a common denominator for the various precipitating factors suggested reduced filtration rate, extracellular fluid expansion and positive sodium balance Possibly an increased demand of oxygen (decreased perfusion increased work) might be a driving force in the release of natriuretic factor The present results are compatible with the existence of an additional natriuretic factor that monitors the basal reset of glomerular tubular balance for sodium during low filtration rate

When the ability to eliminate a rapidly induced positive change in sodium balance was tested the patients with low filtration rates demonstrated a significantly slower sodium excretion than the patients with high filtration rates This was found following both oral and intravenous loads of sodium When an oral load of sodium was given the elimination rate could be directly correlated to the filtration rate The oral test was thought to be the most physiological stimulus of the factors controlling sodium homeostasis It was also found to be an easily accomplished and valuable clinical test for the control of sodium homeostasis

The intravenous studies enabled determinations of absolute values of filtered and reabsorbed sodium as well as of fractional sodium excretion Thereby more information could be obtained on the nature of the slow sodium elimination in patients with low filtration rates The patients with high GFRs respond to the hypotonic saline load with an inhibition of tubular sodium reabsorption This response is the same that has been observed previously in healthy experimental animals (12) The patients with low filtration rates were unable to decrease the tubular sodium reabsorption during the saline load The slow excretion of sodium load in patients with low filtration rates could thereby be directly attributed to an inability to further suppress tubular sodium reabsorption In contrast to the response to a hypotonic saline load the transition from hydropenia to water diuresis elicited an increase in the urinary sodium excretion in the patients with low filtration rates This was effected not only by the increase in filtered sodium but also by a borderline sig

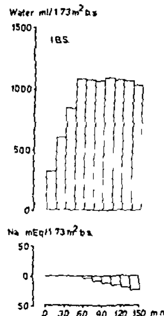


Fig 5 The accumulated changes in water and sodium balance during the transition from hydropenia to water diuresis in patient IBS

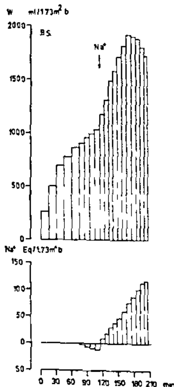


Fig 6 The accumulated changes in water and sodium balance during the course of a hypotonic saline loading experiment in patient BS

nificant increase in fractional sodium excretion Thus the transition from hydropenia to water diuresis must have initiated an inhibition of sodium reabsorption in patients with low GFRs During the transition from hydropenia to wat r diuresis the following changes are accomplished (*a*) a loss of the effect of antidiuretic hormone (ADH) (*b*) a positive water balance is induced with insignificant or negative changes in sodium balance (Fig 5) The loss of ADH effect as such could hardly be exp cted to cause an increase in urinary sodium excretion since in fact the addition of vasopressin itself during water diuresis results in natriuresis (8) Thus it is likely that extracellular volume expansion alone is a stimulus to inhibition of sodium and water reabsorption in patients with low GFRs During superimposed hypotonic saline load the extracellular volume is further expanded and in addition the sodium balance is changed from slightly negative to positive (Fig 6) The lack of further inhibition of sodium reabsorption during superimposed hypotonic saline load might be due to (*a*) the ability to respond to extracellular volume expansion alone is reduced in the patients with low filtration rates as compared to the patients with normal filtration rates and has already reached its maximum during water diuresis and (*b*) the induction of a positive sodium balance in the patients with high GFRs released an additional factor controlling sodium reabsorption In the patients with low filtration rates this factor was either not released or more likely had been released maximally during basal conditions The present data do not allow any further differentiation between those theories

SUMMARY

Basal sodium excretion and rapid response to oral and intravenous sodium loads have been studied in children with recurrent urinary tract infections and a wide range of glomerular fil tration rates Basal sodium excretion related to body surface was remarkably stable in all children studied and thus independent of glomerular filtration rate The rapid response to an oral or an intravenous sodium load was reduced in patients with low filtration rates The urinary sodium excretion rate following oral sodium load was correlated to the glomerular filtration rate with high statistical significance In patients with high filtration rates saline in fusion resulted in a rather prompt inhibition of tubular sodium reabsorption with consequent increase in urinary sodium excretion In patients with low filtration rates inhibition of tubular sodium reabsorption following intravenous sa line load was much less pronounced During the transition from hydropenia to water diuresis without extra sodium supply both the patients with low and with high filtration rates increased the absolute as well as the fractional sodium excretion concomitantly with an increase in the filtered load of sodium. The results are com patible with but do not prove the existence of

an additional natriuretic factor that keeps the basal sodium excretion constant in the diseased kidney

ACKNOWLEDGEMENT

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THE INFLUENCE OF OROTIC ACID ON THE SERUM BILIRUBIN LEVEL OF MATURE NEWBORN

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By the application of enzyme inductors we succeeded in effectively decreasing the serum bilirubin level of premature and mature infants and in counteracting the development of a hyperbilirubinemia Unfortunately however the inductors so far tested have certain disadvantages that prohibit their clinical application or would restrict it The application of phenobarbital (4 17 19 20 22) is not indicated in premature infants with disorders of respiration because of its depressive effects on respiration (10) The application of phenylbutazone (5) causes edema to develop Moreover a displacement of the bilirubin from its protein bond in the serum is likely to occur (1) so that its clinical administration cannot be advised The effect of a therapeutically justifiable dose of nikethamide is too small to achieve a decisive clinical advantage (11)

There are indications that in addition to a not yet matured glucuronyl transferase activity which can be stimulated by inductors a deficiency of the pertinent coenzyme UDP also limits the formation of glucuronyl in the new born child (14 15 23 24) It was pointed out by Brodersen (2) that the synthesis of the UDP takes place via the phase of the orotic acid and that an increased formation of the coenzyme is perhaps achieved by the supply of this biogenic substance In this way an increase in the bilirubin conjugation would be possible

Setting out from this conception we at first tried to decrease the serum bilirubin level in premature infants by the application of orotic acid (6 12) From the clinical point of view as well as for theoretical considerations we took an interest in the question whether the effect of orotic acid in decreasing bilirubin would likewise be detectable in the mature newborn child We therefore carried out adequate investigations the results of which are submitted in this paper

METHOD

At a neonatal unit consisting of four wards 52 mature newborn in two wards were given 200 mg of orotic acid (trial product of VEB Jenapharm Jena) orally in two single doses daily from their 1st to their 5th day of life Fifty newborn children in the other two wards within the same test period served as a control group The average birth weight of those of the newborn who were treated with orotic acid was 3 470 g and of the newborn from the control group 3 465 g

We included in the test and control groups only clinically inconspicuous babies whose mothers had not been given any medication during parturition No children from rhesus immunized mothers were included in either the test group or the control group In both groups the newborn were first given mother s milk 6 hours after birth the quantity of milk then increasing daily by 50 g On their first 3 days of life an additional feeding of a tea glucose mixture up to a total quantity of 200 g was administered In cases of hypogalactia the feeding was done in the same mode with pooled mother s milk from the lactarium

From the 3rd till the 5th day of life the determination of the total and indirectly reacting bilirubin

an additional natriuretic factor that keeps the basal sodium excretion constant in the diseased kidney

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id crease of the serum bilirubin level whereas its administration to premature infants did We came to the same conclusion after increasing the daily dose of orotic acid to 600 mg Matsuda & Shirahata (16) administered a daily dose of 200 mg of orotic acid and found only a slight decrease of the serum bilirubin level in 16 mature infants in comparison with a control group

As to the mode of action of the orotic acid we assume a substitution effect on the coenzyme of the glucuronyl transference We should like to interpret our results as an indication of the fact that this substitution effect becomes effective only in premature infants with a decreased concentration of glucuronic acid No influence can be observed in mature infants with an approximately normal level of glucuronic acid Only in the condition where the not yet fully developed glucuronyl transferase activity of these mature infants might be developed to its full efficiency by enzyme induction could a consequent relative deficiency of coenzyme possibly be remedied by administration of orotic acid

We are presently examining this question in connection with a combined administration of enzyme inductor and orotic acid to mature newborn infants

It is a matter for discussion whether an enzyme inducing effect should be attributed to the orotic acid itself This possibility is disproved by electron microscopical examinations of the liver in the course of which no increase in the endoplasmatic reticulum was detectable after administration of orotic acid as well as by the determination of the liver dependent coagulation factors the activity of which showed no increase during administration (7) Animal experiments are in agreement with these results (13) After all the results given in this paper have to be assessed in a like manner If orotic acid possessed an inductor property it would also have to be detectable in the mature newborn child as is known of other enzyme inductors

The question remains open whether or not the orotic acid has a choleretic effect in the premature infant On the one hand there are indications that the elimination of bilirubin from the liver cell into the gall is decreased in the premature infant (21) on the other hand a choleretic effect of orotic acid was found by Charbonnier & Sagon (3) The absence of any influence on the bilirubin levels can be taken as proof that there is no such effect in mature infants

We did not observe any side effects of the orotic acid in mature infants nor were these to be expected on account of our examinations carried out with premature infants (7)

SUMMARY

Fifty two mature newborn were treated with a daily dose of 200 mg of orotic acid from their 1st to their 5th day of life An equally large number of untreated children served as a control group Contrary to the premature infants in the mature newborn no decrease in the serum bilirubin was achieved by administration of orotic acid The question of the possible mode of action of the orotic acid is discussed

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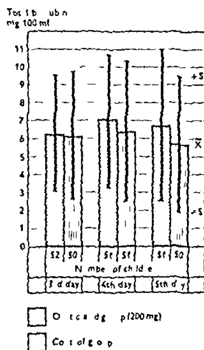


Fig 1

was carried out after the method of Jendrassik et al (8 9)

RESULTS

Arithmetical average value and standard deviation of the total and he indirectly reacting serum bilirubin were calculated from the 3rd till the 5th day of life As Figs 1 and 2 show

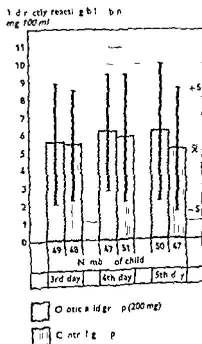


Fig 2

Table 1 *Number of blood exchange transfusions in premature infants after administration of a daily dose of 100 mg respectively 300 mg of orotic acid in comparison with the control group*

	Number of premature infants	Number of blood exchange transfusions	Significance (χ^2 test)
Control group	54	15	$p=5$
100 mg of orotic acid daily	56	6	
Control group	102	30	$p=0.1$
300 mg of orotic acid daily	102	4	

the average values of the serum bilirubin levels of the newborn who were treated with orotic acid are slightly higher than in the control group, but there is no significant difference Blood exchange transfusions were not necessary in either group The bilirubin levels of children that had an existing OA- or OB blood group constellation were watched separately In comparison with the other newborns no higher serum bilirubin levels were registered

DISCUSSION

We administered a daily dose of 100 mg of orotic acid to premature infants from the 1st till the 6th day of life and were thus able to decrease the number of blood exchange transfusions remarkably (Table 1) (6) The bilirubin decreasing effect was even more significant after an increase in the daily dose of orotic acid up to 300 mg Th number of blood exchange transfusions became about a tenth of that of the control group (Table 1) (12) Further investigations are being made to decide the question whether an additional increase in the daily dose of orotic acid is justifiable and whether the bilirubin decreasing effect in the premature child could be further increased in this way

The results of the investigations submitted in this paper show that the application of orotic acid in mature infants did not lead to

a decrease of the serum bilirubin level whereas its administration to premature infants did We came to the same conclusion after increasing the daily dose of orotic acid to 600 mg Matsuda & Shirahata (16) administered a daily dose of 200 mg of orotic acid and found only a slight decrease of the serum bilirubin level in 16 mature infants in comparison with a control group

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REVIEW ARTICLE

ADHESIONS OF LABIA MINORA (SYNECHIA VULVAE) IN CHILDHOOD

A Review and Report of Fourteen Cases

EDEL HARLUND CHRISTENSEN and JAKOB ØSTER

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The terms adhesions of the labia minora or "synechia vulvae denote a condition of complete or partial fusion of the labia minora in the midline The condition has been long recognized and previously considerable interest was shown for it (3 8) In recent years however only superficial mention has been made of the condition even in recognized textbooks of paediatrics (17 20) and it has also received only scant mention in paediatric journals (4 12 14 23 25)

In the majority of cases the condition does not produce any symptoms but occasionally difficulties in micturition and urinary infections may occur Early diagnosis ensures that treatment which is very easy may be undertaken before the patient has experienced somatic or mental repercussions and before the parents for whom it may be a great psychological trauma to discover that their daughter is "abnormal are exposed to unnecessary anxiety A number of parents and several doctors have in fact erroneously suspected girls with this condition of being abnormal most frequently of being pseudohermaphrodites This may best be illustrated by quoting Campbell s example (7) He was consulted by a mother with two daughters aged 5 and 6 years respectively both of whom had vulval adhesions The mother had several doctors and had been told that plastic vaginal reconstruction would be necessary In desperation the mother had made a list of 11 distinguished plastic surgeons from all over USA and intended to consult them all in order to hear about their methods of treatment Campbell immediately undertook simple separation of the labia minora which revealed that both of the girls had normal external genitalia and normal vaginae

It is therefore considered justifiable to draw attention to the condition and to emphasize the significance of thorough investigation of the genital region in girls not only at routine health examinations of children by the general practitioner but also at objective investigations during hospitalization

The authors cases

During the 5 year period 1966–70 the authors have observed and treated 14 girls with adhesions of the labia minora Seven of the patients were admitted on account of other conditions and the vulval adhesions were discovered at routine objective investigation The remaining 7 cases were found by a general practitioner (F Hesselbjerg) at routine medical examinations of healthy children and referred for *outpatient* observation and treatment as he was aware of the authors interest in this condition.

The age distribution of the patients appears in Table 1 which also reveals th extent of the adhesions and other clinical data The various forms of adhesions are illustrated in photographs 1 2 and 3

All of the patients were symptom free In particular

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REVIEW ARTICLE

ADHESIONS OF LABIA MINORA (SYNECHIA VULVAE) IN CHILDHOOD

A Review and Report of Fourteen Cases

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The terms "adhesions of the labia minora or synechia vulvae denote a condition of complete or partial fusion of the labia minora in the midline The condition has been long recognized and previously considerable interest was shown for it (3 8) In recent years however only superficial mention has been made of the condition even in recognized textbooks of paediatrics (17 20) and it has also received only scant mention in paediatric journals (4 12 14 23 25)

In the majority of cases the condition does not produce any symptoms but occasionally difficulties in micturition and urinary infections may occur Early diagnosis ensures that treatment which is very easy may be undertaken before the patient has experienced somatic or mental repercussions and before the parents for whom it may be a great psychological trauma to discover that their daughter is "abnormal are exposed to unnecessary anxiety A number of parents and several doctors have in fact erroneously suspected girls with this condition of being abnormal most frequently of being pseudohermaphrodites This may best be illustrated by quoting Campbell s example (7) He was consulted by a mother with two daughters aged 5 and 6 years respectively both of whom had vulval adhesions The mother had consulted several doctors and had been told that plastic vaginal reconstruction would be necessary In desperation the mother had made a list of 11 distinguished plastic surgeons from all over USA and intended to consult them all in order to hear about their methods of treatment Campbell immediately undertook simple separation of the labia minora which revealed that both of the girls had normal external genitalia and normal vaginae

It is therefore considered justifiable to draw attention to the condition and to emphasize the significance of thorough investigation of the genital region in girls not only at routine health examinations of children by the general practitioner but also at objective investigations during hospitalization

The authors cases

During the 5 year period 1966–70 the authors have observed and treated 14 girls with adhesions of the labia minora Seven of the patients were admitted on account of other conditions and the vulval adhesions were discovered at routine objective investigation The remaining 7 cases were found by a general practitioner (F Hesselbjerg) at routine medical examinations of healthy children and referred for outpatient observation and treatment as he was aware of the authors interest in this condition

The age distribution of the patients appears in Table 1 which also reveals th extent of the adhesions and other clinical data The various forms of adhesions are illustrated in photographs 1 2 and 3

All of the patients were symptom free In particular

Table 1 *Clinical aspects of adhesions of the labia minora in fourteen cases*

Case number	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Age at observation (months)	5	5	6	8	8	12	14	16	17	17	23	24	48	72
Complete adhesion	−	+	+	−	−	−	−	+	−	+	−	−	+	+
Partial adhesion	+	−	−	−	+	+	−	−	+	−	+	+	−	−
Multiple adhesion	−	−	−	+	−	−	+	−	−	−	−	−	−	−
Relapse	−	−	−	−	−	−	−	−	−	−	+	−	+	−

there were no difficulties in micturition no urinary infections nor evidence of vulvitis

Treatment consisted of rupture of the adhesions by means of slight manual traction without anaesthesia The two oldest girls aged 4 and 6 years did not cry or complain during this therapeutic procedure while the smaller girls occasionally cried briefly The impression was obtained that the process could not have been gentler under local or general anaesthesia and that these were not necessary In a single case (Case 8) no treatment was undertaken during hospitalization but the mother was instructed to lubricate the synechial membrane with an indifferent ointment During this procedure the mother perforated the membrane by accident and there has been no recurrence

Following manually produced rupture of the synechial membrane an epithelial defect of the inner surface of the labia minora could be observed but no haemorrhage occurred in any of the 14 cases The mothers were instructed to apply an indifferent ointment to the wound edges thrice daily for a week and thereafter to inspect and wash the external genitalia daily in the subsequent months

All of the patients were followed up in view of possible recurrence after periods of observation which varied from 1 $^{1}/_{2}$ to 24 months (average 7 $^{1}/_{2}$ months) after treatment Recurrence occurred in two girls and as far as could be judged this was because the mother had not carried out the above mentioned therapeutic programme

DISCUSSION

1 *Pathological anatomical conditions*

Fusion between the labia minora consists of an extremely thin, transparent membrane which is commonly thicker posteriorly According to Finlay (10) the adhesion not only consists of fusion of the two adjacent edges of the labia minora but frequently involves the inner surface of the labia This was also observed in the 14 patients of this material in whom the membrane was in all of the cases and in its entire extent, so thin that suitable lateral traction sufficed to complete the rupture

According to several authors (7, 15) the most frequent form is the complete adhesion in which fusion ceases a few millimetres posterior to the clitoris but anterior to the external urethral meatus so that an opening remains through which micturition can occur In occasional cases one or two openings may be present posteriorly in the membrane Nowlin et al (15) observed conditions such as these in 47% out of their 110 patients Finally, adhesions may be partial and in such cases frequently corresponding to the posterior $^{1}/_{2}$ or $^{1}/_{3}$ of the labia minora but partial fusion has also been observed anteriorly in the vulva In the present material, complete and partial adhesions occurred equally frequently with 6 cases in each group while only 2 cases of the type with multiple perforations occurred

2 *Age at which the condition is observed*

Observations in the literature appear to be unanimous (5, 10 14 15) that vulval synechia are observed chiefly within the first 2 years of life although the limits were wide (from 2 months to 7 years) This however, gives no information about the time of origin of the adhesions The present authors have made the same observation as the adhesions in the 14 patients concerned were noted for the first time between 5 months and 6 years of age in 12 cases within the first 2 years of life The authors have never observed adhesions in female infants under the age of 5 months

3 *Incidence*

It has not proved possible to obtain any concrete figures for the incidence of vulval synechia The majority of authors state that ad

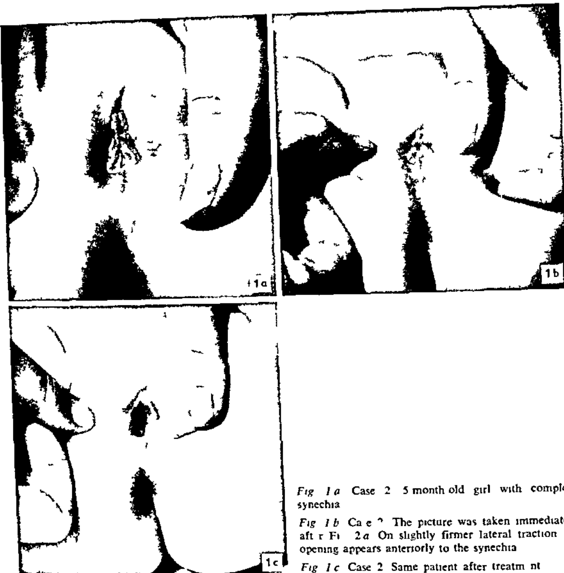


Fig 1 a Case 2 5 month old girl with complete synechia

Fig 1 b Ca e ? The picture was taken immediately aft r Fi 2 *a* On slightly firmer lateral traction an opening appears anteriorly to the synechia

Fig 1 c Case 2 Same patient after treatm nt

hesions occur quite frequently Barysh (2) reports that as a paediatrician he sees from 6–12 cases requiring treatment annually and this is in good agreement with Finlay (10) who also reports having seen the same number of cases annually Finally the material reported by Nowlin et al (15) which originates from an urological and a paediatric department comprises 110 cases observed in the course of 14 years This corresponds to the admission of 7–8 children with vulval synechia annually No information is availabl however concerning the total

Seven out of the 14 patients in this material originate from children hospitalized for other reasons Four of them were found during the year 1969–70 in which all of the girls admitted a total of 287 aged 0–14 years were examined systematically in view of vulval synechia The incidence was thus 1 4% Out of the seven girls referred from general practice 4 were found in 1969 and the other 3 in the first half of 1970 It is strange that no other general practitioner observed any cases They admit however that inspection of the external genitalia is as a rule too superficial



Fig 2 a Case 1 5 month old girl with partial synechia located to the lower half of the vulva cleft



Fig 2 b Case 1 After treatment

4 *Symptoms and complications*

As mentioned previously vulval adhesions rarely cause any symptoms Difficulties with micturition and urinary infections have been described however (2, 13, 18, 19 21), and Nowlin et al (15) found such symptoms in 20% of 110 girls None of the 14 patients in the present material had local or urinary symptoms, and urinary infection was not demonstrated in any case

5 *Etiology and pathogenesis*

In discussion on the etiology of the condition considerable interest has been focused on the question of the condition being congenital or acquired As early as 1825, Dewees (8) maintained that the condition is practically always acquired, a conception which is supported by the majority of authors (4, 15) Campbell (5 6, 7) considers, however, that the condition is congenital but that it is seldom revealed at birth He considers that the congenital form was present in 39 cases It is however not immediately apparent from his statement whether a personal observation or an opinion is concerned The only definite case with congenital vulval synechia was described by Bowles & Childs (4) out of a total of 20 cases observed The problem is most convincingly illustrated by Finlay (10) who examined all newly born female infants in an English maternity department in view of both vulval synechia and congenital dislocation of the hip Out of a total of 5 000 newly born female infants he did not encounter any congenital cases of the former condition

Dewees (8) considered that inflammation of the mucous membrane of the labia resulting from poor hygiene might be the cause of the fusion There can hardly be any doubt that fusion of the mucous membranes may occur in connection with prolonged inflammatory conditions (25) although it is far from proven that this is as a rule the cause of the condition described here Some authors consider that slight cases of vulvitis (8 22 23) or mechanical irritation (4 10) possibly in connection with the great cellularity and vascularity in the labia minora (12 15) may be of significance in the pathogenesis





Fig 3 a Case 7 14 month old girl with an opening both anteriorly and posteriorly

Fig 3 b Case 7 After treatment

Anderson (1) refused however to accept this explanation He maintained that he had never observed any connection between genital hygiene and the occurrence of synechia and added that a possible explanation for the pre sence and recurrence of these adhesions is that the *mucus* secretions in such girls contain a certain musilaginous property that causes these two opposing surfaces to adhere This is pure speculation

Finally Teton (22) in 1956 mentioned that adhesions of the labia minora via a non specific vaginitis might be "due to an exaggeration of the normal hypoestrogenism of childhood

Williams & Cramm (24) agreed with this conception which may also explain why the condition is not observed in newly born infants and is rare in women after the *menarche* (6 12) as increase in the circulating oestrogen oc curs in both of these age groups *The condition* has however been described in adults (21) Finally Dewhurst (9) considered that this theory was supported by the observation that oral administration of oestrogen appears to re sult in disappearance of the adhesions This entire question seems to be very debatable and no investigations of oestrogen levels in girls with vulval adhesions are available

The present authors have never observed synechia in newly born infants and vulvitis could not be demonstrated in any of the 14 cases reported *here*

6 *Treatment*

In his early investigations Dewees (8) pointed out that the condition may disappear spon taneously and this has since been supported by other authors (1 10) The majority of au thors *still* maintain that no treatment is neces sary for small partial adhesions Complete or extensive partial synechia and cases causing *local* symptoms or urinary infections should be *treated The majority of authors* consider that the membrane should be incised or divided in the mid line (2 5 8 11) As mentioned pre viously *Teton* (22) considered that the condi



Fig 2 a Case 1 5 month-old girl with partial synechia located to th lower half of the vulva cleft



Fig 2 b Case 1 After treatment

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tion could be treated successfully by oral ad ministration of oestrogen while Williams & Cramm (24) found that local application of oestrogen ointment had an equally good effect

Treatment such as this may appear drastic and unnecessary for a condition which as in the 14 cases presented here, may be treated without undue discomfort by lateral manual traction of the labia majora which causes the membrane to rupture without haemorrhage

7 *Prognosis*

It is apparent not only from the literature (5, 16 22) but also from the authors experience that follow up treatment for a week or more with application of an indifferent ointment to prevent the formation of new adhesions between the raw mucosal surfaces, is very im portant Nowlin et al (15) found, that despite this procedure one or more recurrences took place in 20% of their 110 patients In the present series recurrence was observed in two out of 14 patients i e, approximately the same proportion (14%) but it was obvious that these 2 cases occurred because the follow up proce dure and control were defective No untoward psychological effects attributable either to the condition or to the treatment have been ob served in the children All of the parents expressed their satisfaction that the treatment was so easy and their relief that the child was now normal

SUMMARY

The diagnostic and therapeutic problems in 14 cases of adhesions of the labia minora in chil dren are mentioned It was found that the con dition is quite frequent, is acquired and occurs predominantly during the first 2 years of life

Thorough investigation of the external genitalia in little girls is essential to establish the diagnosis This is important because the treat ment, in which the adhesions are ruptured by means of slight lateral traction, is very easy and painless and because the untreated condition may result in urinary symptoms Follow up treatment with an indifferent ointment applied to the exposed epithelial surfaces is important in order to avoid recurrence

Recognition of the condition is important as incorrect interpretation as pseudoherma phroditism may cause much anxiety and trouble for the children and their families

The pathogenesis is unknown and the hypo thesis that it is due to an exaggerated form of the normal childhood hypoestrogenism in the affected children deserves further investigation

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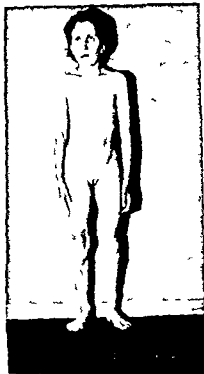


Fig 1 The patient aged 16 years and two months.

somes showed a *section of* bright fluore cence on the distal egment of the long arm (Fig 4) The analysis of even c lls from skin cultures showed five of them to have 45 chromosomes (consistent with an X0 constitution) and two 46 chromosomes The analysis of nine cells from cultures of Fallopian tube tissue showed in four of them 46 chromosomes and in five 45 Both in skin and in Fallopian ube culture the cells with 46 chromosomes had five achro c ntrics as in p ripheral blood cultures

COMMENT

The presence of short stature mental retardation widely spaced nipples skeletal alterations such as hypertrophy of the medial condyle of the tibia and small pituitary fossa and other clinical minor abnormalities in a subject with sexual hypoplasia and streak gonads suggested the diagnosis of gonadal dysgenesis with many of the features of Turner s syndrome Blood lymphocytes showed 46 chromosomes with five small achrocentrics One of these although a little larger could not be interpreted as a Y on its morphological ap p arance alone The autoradiography was not informative No hot chromosome was found among 40 labelled metaphases and this finding suggested that the abnormal chromosome was not an X fragment Identification of this chromosome was achieved by applying fluorescence technique to peripheral blood cultures We demonstrated a fluorescent section corresponding to the distal segment of the long arm of one of the five achrocentrics this phenomenon has been recently demonstrated to be characteristic of the Y chromosome (8 6) A sex chromosome complement of the XY

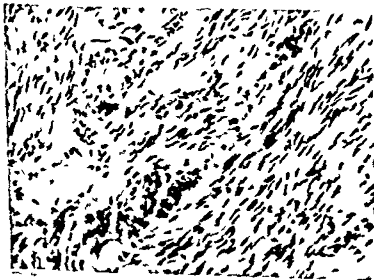


Fig 2 Histology of the biopsy fragment from the right gonad

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CASE REPORT

IDENTIFICATION OF THE Y CHROMOSOME BY THE FLUORESCENCE TECHNIQUE IN AN XY/X0 GONADAL DYSGENESIS

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From the Paediatric Clinic of the University of Pavia the Euratom Unit for Human Radiation and Cytogenetics and Institute of General Biology of the Medical Faculty University of Pavia Italy

We report in this paper a case of gonadal dysgenesis with XY/X0 chromosome sex complement The Y chromosome was definitely identified with the aid of the fluorescence technique

CASE REPORT

Gianna R this Italian girl was born on April 25th 1954 when her mother and father were respectively 24 and 26 years old She was the product of a normal pregnancy labor and delivery The mother did not take any drugs neither was she exposed to radiation during pregnancy The child s birth weight was 3 250 g She was the first of three siblings Two sisters are normal The parents are not consanguineous They had observed that the patient s growth was stunted from her second year of life and later that she was slightly mentally retarded At the age of 14 years she weighed 25 kg and was 128 cm in height corresponding to a height age of 8 years and 8 months (1) Signs of sexual development were absent At the age of 16 years and 2 months (16 6 1970) she was admitted to the Paediatric Clinic of the University of Pavia for investigation of dwarfism and sexual infantilism

Physical examination showed a patient of definite female phenotype but with a development corresponding to an age of about 10 years (Fig 1) Weight 27 kg height 133 cm height age 9 years and 8 months (1) lower segment 67 cm upper/lower ratio 0 98 span 139 cm head circumference 54 cm chest circumference 68 cm Hypertelorism and slight arched palate were present The auricles were large but not low set and the occipital hairline slightly low The neck was short but not webbed Breast development was absent and the nipples were widely spaced Slight cubitus valgus was present External genitalia were morphologically normal but underdeveloped little sexual hair was present on the labia majora Heart sounds were normal as were the electrocardiogram and the phonocardiogram Liver and spleen were not palpable Fundus oculi was normal The Wechsler Intelligence Scale core was 61 Skeletal X ray examination showed a skeletal age of 11–12 years (2) normal metacarpals hypertrophy of the medial condyle of the tibia Parietal and frontal bones were thickened and the pituitary fossa was small A pyelogram showed a normal urinary tract A vaginogram showed the existence of a vagina about 4 cm in length The urinary oestrogens excretion was 2 6 μg/24 hrs and the urinary 17 ketosteroids excretion was 4 mg/24 hrs The urinary gonadotropins excretion fluctuated between 336 and 800 HMG IRP II/24 hrs on twelve consecutive days Dermatoglyphs were normal Endoscopic examination by Wolff s photocoelioscope showed a very hypoplastic uterus and short and thin Fallopian tubes Gonads were represented by two club shaped streaks with a smooth surface Biopsies were taken from the right gonad (for histologic examination) the right tube and the skin (for chromosomal study) On histological examination the right streak gonad consisted of abundant connective tissue compressing residual epithelial cords (Pflüger s cords) and was completely devoid of follicles (Fig 2)

Sex chromatin was absent in a buccal smear The examination of 46 cells from blood cultures showed in all of them 46 chromosomes with five small achrocentrics (Fig 3)

Autoradiography was performed labelling a blood culture for the last four hours with 0 5 μCi ml of tritiated thymidine (Amersham s a 6 Ci mM) No obvious late replicating chromosome was found in 40 labelled metaphases analysed *Fluorescence* technique was applied to slides from blood cultures stained with quinacrine dihydrochloride according to Pearson et al (6) One of the five small achrocentric chromo

whereas ovarian follicles have been clearly demonstrated only in the case reported by Hirschhorn (4 5) This finding agrees with the hypothesis that an XY/X0 mosaic arises from the loss of a Y chromosome during an early mitotic division of an XY zygote Thus far it is not possible to establish if the variety of clinical pictures and of gonadal alterations is related to the relative proportion of cells with 45 and with 46 chromosomes in various tissues Finally as far as our case is concerned the clinical features and the histological findings are in agreement with a diagnosis of Turner s syndrome the caryotype was established as an XY/X0 mosaic in which the Y chromosome has been identified by the fluorescence technique This technique allows an easy identification of the Y chromosome in cases like the present one in which a differentiation between the Y chromosome and X fragments is needed

SUMMARY

A phenotypic female aged 16 years had sexual hypoplasia streak gonads and sex chromosome mosaicism She was XY in peripheral blood cultures and XY/X0 in Fallopian tube tissue and in skin cultures A definite identification of the XY line was made by the fluorescence technique which showed a fluorescent section corresponding to the distal segment of the long arm of one of the five achrocentrics

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Fig 3 Karyotype of a cell from peripheral blood culture with 46 chromosomes

type in a female phenotype has been usually found in pure gonadal dysgenesis and only in very few patients with the features of Turner syndrome (3) We therefore suspected the existence of a tissue mosaic which was confirmed by the chromosomal analysis of the Fallopian tube tissue and of the skin which were found to be XY/X0

This type of mosaicism corresponds to a large variety of gonadal abnormalities and of clinical pictures It was reported chiefly in mixed gonadal dysgenesis but also in male pseudohermaphroditism in the so called 'male Turner syndrome' and in few cases of Turner s syndrome According to Van Campenhout (7) out of 51 cases with XY/X0 sex chromosome complement and with bilateral gonadal identification 38 had testicular tissue,



Fig 4 Details of three cells from peripheral blood culture stained with quinacrine dihydrochloride showing a fluorescent section on the distal portion of a small acrocentric chromosome

whereas ovarian follicles have been clearly demonstrated only in the case reported by Hirschhorn (4 5) This finding agrees with the hypothesis that an XY/X0 mosaic arises from the loss of a Y chromosome during an early mitotic division of an XY zygote Thus far, it is not possible to establish if the variety of clinical pictures and of gonadal alterations is related to the relative proportion of cells with 45 and with 46 chromosomes in various tissues Finally as far as our case is concerned the clinical features and the histological findings are in agreement with a diagnosis of Turner s syndrome the caryotype was established as an XY/X0 mosaic in which the Y chromosome has been identified by the fluorescence technique This technique allows an easy identification of the Y chromosome in cases like the present one in which a differentiation between the Y chromosome and X fragments is needed

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CASE REPORT

CONGENITAL LEUKAEMIA

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Leukaemia in the newborn infant is extremely rare The clinical picture in this age group deviates on several points from that of leukaemia developing later in life The disease is manifest even during foetal life and, consequently, several interesting aetiological aspects are associated with this disease In this paper one case of congenital leukaemia will be discussed on the basis of these considerations

CASE REPORT

In april 1969 a newborn boy was transferred from the maternity ward to the paediatric department of the Glostrup Hospital because of wide spread haemorrhages in the skin The mother was 20 years old she was pregnant for the second time and had two years earlier given birth to a normal girl The mother had always been healthy and was still so at the follow up in July 1970 There was no family history of malignant diseases Pregnancy had been uncomplicated without exposition of the mother to roentgen examination drug treatment or infectious diseases Delivery after puncture of the membranc was normal Birth weight 3 150 g length 52 cm The placenta was normal Apgar score 3 after one minute and 7 after 10 min

Minor cutaneous infiltrations reminding of tumour dissemination were seen immediately at birth as well as haemorrhages in the skin (Fig 1) There was moderate swelling of the liver and spleen and the inguinal lymph nodes were the size of beans The general condition of the infant was surprisingly good

Some of the results obtained by examination of peripheral blood and bone marrow are recorded in Table 1 The blood showed marked leucocytosis with many promyelocytes myelocytes and stem cells (Fig 2)

Other examinations The bleeding time exceeded 10 min Blood urea 0 42 g/l GP transaminase alkaline phosphatase serum protein electrophoresis and immuno electrophoresis of serum normal findings The blood contained no thrombocyte antibodies and the titre of cytomegalo virus antibodies was normal The parotitis complement binding reaction was negative Culture from the bone marrow showed growth of staphylococcus albus (probably contamination) but culture from blood and spinal fluid was negative Urine microscopy was normal without owl eye cells ECG normal Roentgenograms of the chest long tubular bones the skull the pelvis and the spine was normal Ophtalmoscopy was normal Chromosome investigation was attempted on peripheral blood and bone marrow from mother and child but without success

In *the maternal blood* the following were normal Hb leucocyte and thrombocyte counts serum electrophoresis cytomegalo-virus antibody titre parotitis complement binding reaction Wassermann reaction Biopsy of the bone marrow showed nothing abnormal Alkaline phosphatase 8 (decreased) No owl eye cells in the urine IgA and IgM in the serum were lowered and haptoglobin was increased

Despite treatment with parenteral fluid hydrocortisone and vincristin® the patient died on the 7th day of life

Autopsy No malformations were found Leukaemic infiltrations were seen in the lymph nodes bone marrow lungs liver spleen kidneys and pancreas (Fig 3)

DISCUSSION

:e Pollmann (15) in 1898 described the t case about 50 cases have been reported,



Fig 1 Cutaneous infiltrations in a new born boy with congenital leukaemia

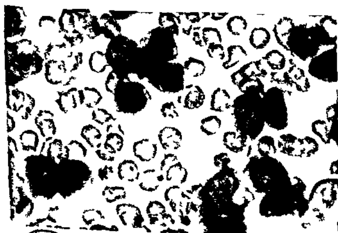
Table 1 *Results of differential count in blood and bone marrow*

	1st day of life		4th day of life	
	Marrow	Blood	Marrow	Blood
Hb g 1		116		138
Thrombocyte count/µ l		79 000		67 000
Leucocyte count µ l		311 000		352 000
Hemocytoblasts (Stem cells)	76	25	76	70
Pr myelocytes	43	38	2	4
Myelocytes	11	18	1	3
Metamyelocytes	6	7	3	4
Neutrophil granulocytes	1	7	12	15
Lymphocytes	2	3	3	2
Basophil erytro-blasts	9	—	—	—
Polychromatophil erythroblasts	1	1	—	—
Normoblasts	1	1	3	2
Total count	100	100	100	100

but some of these appear doubtful The cases have occurred without any geographic accumulation except that a rather high number have been reported from Scandinavia (2 6 9–12 18 19) It has been attempted to classify congenital leukaemia into various types according to the time of onset of symptoms (4) According to Dalgaard & Kaass (6) the disease should only be considered congenital if the symptoms or clinical findings are manifest immediately at birth or within the first week of life

The diagnosis is not correct unless immature leucocytes and stem cells are present not only in the ordinary extramedullary haemopoietic organs but also in non haemopoietic organs (4 14 17 18)

Congenital leukaemia is generally of the myeloid type (80 %) as a rule it is associated with marked leucocytosis in which promyelocytes predominate both in the peripheral blood and in the bone marrow Some cases however must be classified as stem-cell leukaemia Anaemia which rarely is manifest at birth de



Fi 2 Peripheral blood

Acta Pædiat Scand 60 720–723, 1971

CASE REPORT

CONGENITAL LEUKAEMIA

J HAAHR and A B HALVEG

From the Paediatric Department and the Pathological Institute Copenhagen County Hospital Glostrup Denmark

Leukaemia in the newborn infant is extremely rare The clinical picture in this age group deviates on several points from that of leukaemia developing later in life The disease is manifest even during foetal life and, consequently, several interesting aetiological aspects are associated with this disease In this paper one case of congenital leukaemia will be discussed on the basis of these considerations

CASE REPORT

In april 1969 a newborn boy was transferred from the maternity ward to the paediatric department of the Glostrup Hospital because of wide spread haemorrhages in the skin The mother was 20 years old she was pregnant for the second time and had two years earlier given birth to a normal girl The mother had always been healthy and was still so at the follow up in July 1970 There was no family history of malignant diseases Pregnancy had been uncomplicated without exposition of the mother to roentgen examination drug treatment or infectious diseases Delivery after puncture of the membrane was normal Birth weight 3 150 g length 52 cm The placenta was normal Apgar score 3 after one minute and 7 after 10 min

Minor cutaneous infiltrations reminding of tumour dissemination were seen immediately at birth as well as haemorrhages in the skin (Fig 1) There was moderate swelling of the liver and spleen and the inguinal lymph nodes were the size of beans The general condition of the infant was surprisingly good

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Autopsy No malformations were found Leukaemic infiltrations were seen in the lymph nodes bone marrow lungs liver spleen kidneys and pancreas (Fig 3)

DISCUSSION

Since Pollmann (15) in 1898 described the first case about 50 cases have been reported

influenced by one or several genes localized in chromosome 21 Except for these studies the occurrence of leukaemia in newborn infants does not give any clue to the aetiology

SUMMARY

A report is submitted of a case of leukaemia of myeloid type in a newborn boy who died on the 7th day of life, despite anti leukaemic therapy The available reports on congenital l kaemia and on children born of mothers with leukaemia in pregnancy do not give any clue to the aetiology

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Key words Congenital leukaemia



Fig 3. Leukaemic infiltrations in the pancreas.

velops shortly after, while thrombocytopenia usually is found from the beginning. Cutaneous and mucosal haemorrhages, together with swelling of the liver and spleen, are common clinical findings, whereas lymph node swelling is rare. Nodular skin infiltrations is a characteristic finding.

The differential diagnoses include leukaemoid reactions attributable to sepsis, congenital syphilis, erythroblastosis and congenital thrombocytopenic purpura.

Treatment has so far been without effect and generally death occurs within few days (4), although a few cases surviving several months have been observed; the latter are seen mainly among patients with Down's syndrome (7, 8, 13).

The aetiology of congenital leukaemia is just as obscure as that of other types of leukaemia. The case histories hitherto reported fail to provide any data concerning factors commonly considered responsible (14). Among the mothers to children with congenital leukaemia none had leukaemia during pregnancy. One case is on record, however, in which leukaemia was diagnosed in the mother immediately after parturition, and acute lymphatic leukaemia developed nine months later in the infant (5). Generally, congenital leukaemia must be induced by factors which do not affect the mothers. An intra uterin factor seems to inhibit the leukaemic process in the fetus, since the symptoms often are slight immediately after birth but aggravate rapidly afterwards (14). If a virus were assumed to be involved, it must act upon the child without affecting the mother but apparently this does not agree with the well known fact that mothers affected with leukaemia during pregnancy give birth to healthy children (3). If hereditary factors were involved, an increased incidence of leukaemia among children born of leukaemic mothers might be expected, but this is apparently not the case (1).

It is a well-documented fact that the incidence of leukaemia in patients with Down's syndrome is higher than that in the general population. The same applies to congenital leukaemia: in a series of 45 cases, 10 were found in Down's syndrome (13). It is of interest that patients with chronic myeloid leukaemia usually present with a chromosome anomaly including deletion probably from a chromosome in the G 21 group, the so called Philadelphia chromosome. In these patients, the alkaline phosphatase level in leucocytes is rather low in contrast to the findings in patients with Down's syndrome of the trisomy-21 type in whom the level of alkaline phosphatase in leucocytes is elevated (4, 16, 20). Even though this is not fully elucidated chromosome studies so far suggest that the leucopoiesis is

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Fig 3 Leukaemic infiltrations in the pancreas

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where children also often complain of stomach pains

On the other hand the gastrointestinal symptoms have whatever their origin an emotional effect on the child and on his parents Psychotherapy of children with repetitious stomach pains requires a very close collaborative treatment of the mother often with the same therapist

O Koskimies

Acta Pædiat Scand 60 724–725, 1971

PROCEEDINGS OF PAEDIATRIC SOCIETIES

THE FINNISH PAEDIATRIC SOCIETY

Meeting April 3, 1971

K Launiala & J K Visakorpi *Recurrent abdominal pain in children A review of somatic aspects*

Recurrent abdominal pain (RAP) is well known to be common in children of the age of 5–15 the prevalence being an average of 10–12 % A history of abdominal complaints in the family and symptoms such as sleep disorders nausea headache dizziness pallor and cold and sweaty hands seem to be common in children with RAP In clinical examination an organic disorder is found in only 4–8 % of children with RAP, the main disorders being urogenital malformations and infections duodenal ulcer and gallstones in pubescent girls In these cases the type and localization of the pain is often different from typical transient umbilical pain in children without organic disorders Recently acquired lactose malabsorption has been shown to be present in many populations of school age children (the prevalence being for example 6 % in Finland) and to be the main cause of abdominal complaints (diarrhoea meteorismus pain) in milk drinking children with this disorder

In the diagnosis and treatment of children with RAP excluding organic diseases is most important With careful history and with clinical examination which includes routine laboratory tests it is often possible to exclude anaemia, occult bleeding in the intestine and urinary abnormalities X ray examinations should be performed only if the type and localization of the pain indicate some organic disorder, intravenous pyelography then often being most informative To exclude lactose malabsorption a per oral lactose tolerance test is suggested

Maija Liisa Koski *Recurrent abdominal pain in children Psychiatric aspects*

Somatic responses influenced by emotions can roughly be divided into two categories temporary dysfunctions related to short lived emotions and more lasting functional and structural changes related to long standing emotional conflicts

Earlier theoretical concepts of the interrelationships between emotions and diseases centred around two specificity theories Dunbar s theory of specific personality and Alexander s theory of specific conflict Later the trend has been away from the specificity theories towards more general stress theories

Gastrointestinal disturbances have been the centre of psychosomatic interest In childhood repetitious stomach pains are seen in many different conditions Colic is one of the earliest gastrointestinal disturbances The role of constitutional factors has been stressed in producing colic On the other hand psychological factors are to be found It is more common in the eldest child of the family and is almost never seen in hospital Therefore it is thought to be related to the mother s tension Aerophagia is seen in somewhat older children and can persist until adulthood Anorexia nervosa peptic ulcer mucous and ulcerative colitis are among the conditions in which repetitious gastrointestinal pains ocur Frustrated dependency needs have been viewed as the key problem in these conditions as well as in school phobia

tracycline and erythromycin All of the children were over the age of 2 years With one exception good agreement was present between the radiological and the clinical and stethoscopic findings

This work is part of investigations published on a previous occasion on the incidence of m p infections among patients admitted to hospital with respiratory infections

At present insufficient facilities are available for routine investigation for m p antibodies in Denmark It is recommended that all cases of protracted pneumonia with positive cold agglutinin titres be suspected to be caused by m p when the common causes of infection have been excluded This cause is particularly possible when the patients have in addition increase in the streptococcus MG antibody titre as it appears that an antigen relationship exists between the two organisms

Discussion

Carl Friderichsen Unproductive cough is very characteristic in m p pulmonary infections
Jørgen Kringelbach The stethoscopic findings in primary atypical pneumonias are more pronounced than the radiological findings
Erik Thamdrup Was culture for virus undertaken?
Jørn Møller No
Erik Bamberg Were any characteristic pulse changes present?
Jørn Møller No

Jørgen Bent Andersen *The effect of diuretics administered during the latter part of pregnancy on newly born infants*

Alterations in the water and salt relationships were investigated in a number of newly born infants the mothers of whom had been treated with one of the two diuretics Chlorthalidone (Hygrotone®) and Bendroflumethazide (Centyl®) during the latter part of pregnancy and until delivery Indications for the diuretic therapy were tendency to oedema and/or abnormal increase in weight but cases of pre eclampsia and eclampsia were excluded from the material

In the infants the following were investigated 2–4 hours after delivery total body fluid extra-cellular volume serum sodium serum potassium serum chloride and total osmolality These values were compared with the values in a group of newly born infants whose mothers had not received diuretic therapy All six parameters had undergone changes compatible with a diuretic effect on the foetus The total fluid extra-cellular volume serum sodium serum chloride and osmolality were lowered while the serum potassium had increased The alterations were slight but the investigation demonstrated that diuretics administered to pregnant women exert a diuretic effect on the foetus also

Discussion

N J Brandt Is the physiological loss of weight identical in infants of mothers treated with diuretics and infants of mothers who had not received this form of treatment?
Bent Andersen The material is too limited to assess this

Meeting Nov 11 1970

Th Rosendal *Radiographic changes in acyanotic heart disease in infants*

Determination of the volume of the heart/m² body surface by means of the formula for the volume of an elipsoid $\left(v = \frac{4}{3} \pi \frac{l}{2} \frac{b}{2} \frac{d}{2}\right)$ on the basis of the longitudinal transverse and vertical diameters as measured by X ray photographs of the thorax in the sitting posture and following correction for enlargement showed in 559 infants with verified congenital heart disease of 31 different types increase in vol

Acta Pædiat Scand 60 726–732, 1970

PROCEEDINGS OF PAEDIATRIC SOCIETIES

THE DANISH PAEDIATRIC SOCIETY

Meeting Sept 9, 1970

Kristine Hauge Kristensen, Knud E Petersen & Erik Thamdrup *Acute suprarenal cortical insufficiency in infancy A case of congenital isolated hypoaldosteronism*

A male infant aged 3 weeks was admitted on account of failure to thrive Pregnancy and delivery had been normal On admission the weight was 230 g below the birth weight and the infant was dehydrated with hyperkalaemia and hyponatriaemia A brother and an uncle had died in infancy with similar symptoms

Following a review of the differential diagnostic possibilities in cases of salt loss in infancy it was considered that the cause was not to be found in the kidneys although the child had pyuria and vesico ureteric reflux Congenital hyperplasia of the suprarenal cortex (adrenogenital syndrome) with salt loss could be excluded on account of the normal excretion of 17 ketosteroids and the absence of pregnantriol in the urine Congenital hypoplasia of the suprarenals was another possibility The clinical picture corresponded exactly to the symptoms exhibited by the children with isolated hypoaldosteronism described by Visser, inter alii Investigations were therefore rapidly focused on this

Normal cortisol production (secretion rate) with response to ACTH was encountered while the aldosteron production was practically non existent and did not respond to pronounced salt loss despite adequate stimulation via the angiotensin–renin system Corticosterone production was greatly increased as evidence of a block in biosynthesis between corticosterone and aldosterone Future investigations will reveal whether a defect in 18 hydroxylase or in 18 hydroxy dehydrogenase is concerned

Treatment of these patients consists of administration of sodium chloride supplements and desoxy corticosterone It is emphasized however that there is a tendency for the salt loss to diminish during the first years of life so that treatment should be followed up and possibly stepped down Apart from the above mentioned symptoms, the infant appeared to have brain damage with retarded psychomotor development

Meeting Oct 14 1970

Jørn Møller *Infections with mycoplasma pneumoniae in children*

A review of the infectious qualities of mycoplasma pneumoniae (m p) is followed by a report of 5 cases of pneumonia probably caused by this organism Isolation of the organism in cultures from throat swabs and four fold increase in the titre of antibodies to m p are greatly suggestive of these infections It is emphasized that m p grows very slowly even under optimum conditions in vitro In all of the cases increase in the titres of cold agglutinins were demonstrated The infections ran protracted courses and appeared to react to

tetracycline and erythromycin All of the children were over the age of 2 years With one exception good agreement was present between the radiological and the clinical and stethoscopic findings

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ume of over 300 ml/m² (normal values for infants) in 75 per cent and up to the double in one third of the cases while the cardio thoracic index was only found to be increased to more than 0 65 (normal value) in 20 per cent Determination of volume thus permits more accurate determination of whether the heart is pathologically enlarged than measurements of the cardiac ratio and it is of diagnostic value

The greatest increases in volume are encountered in fibro elastosis and coarctation of the aorta and in cases of large left–right shunt (VSD, PDA and ASD) Increase in volume is however, also found in cases of tumour of the pericardium with fluid accumulation and in tumour of the myocardium and subsequently in glycogenosis, diaphragmatic hernia through the tendineum centrum with elevation of a heart of normal size and in cases of tumour of the thymus

Determinations of heart volume do not contribute to the differential diagnosis in 361 acyanotic cases of congenital heart disease of 21 different types in infants but may serve to differentiate between the slighter and more severe degrees of the disease

Where the same group of infants is concerned, the four chambers of the heart the aorta and the pulmonary artery with its branches in the lungs were assessed on the basis of X ray photographs without knowledge of the verified diagnosis Uncertainty in the assessment appears from the observation that in 120 infants with normal heart volume (less than 300 ml/m²) enlargement of one or more chambers was registered in 42 per cent and these were registered as normal in 23 per cent of 353 infants with increased heart volume The reason for this is to be found in the indefinite criteria, the elasticity of the heart and the fact that a combination of different malformations of the heart are frequently present simultaneously

In a series of cardiac diseases in infants in the acyanotic group and particularly in the severe degrees enlargement of a single chamber or combination of several chambers may produce a characteristic radiographic picture For example considerable enlargement of the left ventricle combined in a number of cases, with enlargement of the left atrium in fibro elastosis of the left ventricle and atrium and coarctation of the aorta, increase in width superiorly and to the left is observed in corrected transposition constriction of the pulmonary arch in pulmonary stenosis prominence of the right border of the heart in ASD and VSD and enlargement of the right ventricle with elevation of the heart in VSD and PDA Finally, in cases with aberrant pulmonary veins with enlargement of the right atrium and the right ventricle and large superior vena cava and possibly persisting left superior vena cava along or within the upper border of the heart can produce a shadow resembling a snowman

Variations in the shape of the heart are however so great that alterations in the shape of the heart only provide limited assistance in the differential diagnosis In the largest group of 83 infants with verified VSD with pressure in the right ventricle of greater than 40 mmHg and increased heart volume to more than 300 ml/m practically every possible combination of dilatation of the various chambers of the heart was encountered

Alterations in the vascular shadows in the lungs are of much greater diagnostic significance Reduced vascular markings were shown only in cases of pulmonary stenosis in this series Increased vascular markings were encountered in up to 50 per cent of cases with left–right shunt without alterations of pressure in the right ventricle and up to 89 per cent when the pressure in the right ventricle was increased to more than 40 mm Hg so that this is an important criterion in left–right shunt although it does not render any definite information concerning the localization of the shunt Simultaneously prominence of the pulmonary artery arch suggests ASD or PDA but occurs only in half of the patients with congenital heart disease of these two types

Transition of left–right shunt to right–left

shunt in connection with progression of pulmonary hypertension may become apparent in ordinary X rays as diminution of vascular markings and increasing prominence of the pulmonary arch

Discussion

Bent Friis Hansen How many infants without heart disease have nevertheless enlarged hearts? In practice it is a difficult problem to decide whether an infant with cyanosis or respiratory distress has an organic heart disease or an enlarged heart merely on account of anoxia or anaemia Further it would be interesting to know how many completely healthy children have enlarged heart volumes

Th Rosendal I have no experience of children in the first year of life

J Vesterdal Has the phase of respiration been taken into consideration? When the infant cries great changes can occur in the size of the heart and the degree of filling of the pulmonary vessels When the child presses the heart becomes smaller and the pulmonary vessels empty and the reverse occurs during forced inspiration How can it be ascertained whether the alterations observed in Dr Rosendal s material are genuine?

Bent Friis Hansen Does the position of the patient (lying or sitting) play any part for the determination of heart volume?

Th Rosendal (to J Vesterdal and Bent Friis Hansen) I do not think that heart volume determinations are influenced by the position of the patient Nor do I consider that the phase of respiration plays any part

Ib Boesen The X ray picture of a lying patient is distorted and cannot be employed for determination of volume The phase of respiration is of importance and the picture should be taken in inspiration

S Brandt & B Brammer *Demonstration of four patients with good recovery from infantile spasms and hypsarrhythmia*

A brief introductory review was given of 18 patients treated with steroids in Queen Louise s Hospital for Children during the period 1959–69

Eight of these patients had other serious neurological conditions simultaneously Two of the patients had oligophrenic siblings All of these 10 became mentally retarded

In the remaining 8 patients no known predisposition to neurological conditions was encountered and no neurological conditions other than infantile spasms were present

Four of these patients became mentally retarded although subsequent follow up showed distinct mental progress as assessed by intelligence tests

The remaining patients developed normally both mentally and motorically These patients were demonstrated Three of them had shown psychomotor retardation on admission One of these had gradually regained normal mental status At subsequent tests the IQ was found to increase until it became normal 5 years later

In the remaining patients normal findings were encountered after cessation of steroid treatment

Discussion

J Vesterdal Have there been any new findings concerning the etiology and pathological anatomy? In particular can anything be said about the relationship to whooping cough vaccination?

J C Melchior In reply to the question about the connection between infantile spasms and vaccination reference is made to the work in *Ugeskrift for Læger 131* 746–748 1969

I consider that the vast majority of cases are due to temporal coincidence and a similar opinion was expressed at a meeting of European Child Neurologists in Oxford in September 1970 and supported by participants from 16 countries

Conversely it appears to be impossible to deny that in isolated cases vaccination may be

a cause when it is considered that any form of injurious influence on the CNS at a certain stage of the development may result in the symptom of infantile spasms

As a reply to J Vesterdal who sought information on the etiology and the pathological anatomy, I can refer to the work undertaken by Erna Christensen and myself (*Danish Medical Bulletin 7* 121–127, 1960) in which it was demonstrated that practically every possible form of pathological change may be anticipated in the central nervous system and that the decisive factor is probably a discrepancy between the development of the CNS and the chronological age of the child

The results of these investigations have since been confirmed from numerous other sources and are supported also by subsequent reports of materials from Rigshospitalet

Erik Thamdrup We have now adopted early vaccination for whooping cough Has this altered the incidence of infantile spasms?

J C Melchior We dare not make any statement about changes in the incidence but we have observed infantile spasms in connection with vaccination for diphtheria and tetanus, i e about the age of 5–9 months

B Brammer It is possible that the prognosis is better in patients in whom no other neurological conditions can be demonstrated and who are not predisposed to neurological conditions

Jørgen Haahr *Malformations of the urinary tract in children with urinary infections*

During the period 1 VII 1964 to 31 XII 1968, a total of 95 children were admitted to Queen Louise s Hospital for Children with verified urinary infections By urinary infection is understood here growth of at least 100 000 bacteria/ml in a mid stream specimen of urine and leukocyturia with at least 5 leucocytes per field in the centrifuged urine Malformations were encountered in 68 per cent Vesico ureteric reflux and obstruction of the urinary passages were the most frequent malformations Twenty eight out of the 65 children with malformations had two or more malformations of the urinary tracts

Obstructive lesions of the urethra were suspected in 13 children but verified in 9 cases only Three children had urethral valves two sclerosis of the sphincter, two organic meatal stenosis, one double urethra and one a urethral stricture It is emphasized that more attention should be focused on the lower urinary passages With the possible exception of simple dilatation of the urethra, surgical intervention should, however, be delayed until the obstructive urethral lesion is verified and this should be undertaken on the basis of the total assessment of the results of the various investigations as all of these reveal great physiological variations in normal children

Jørgen Haahr *Vesico-ureteric reflux in children*

In a material from Queen Louise s Hospital for Children consisting of 95 children with urinary infections vesico ureteric reflux was demonstrated in 38 (40 per cent) 25 girls and 13 boys All of these were followed up regularly in the out patient department for a period of observation of approximately 3 years Twenty three of the patients had reflux alone and the other 15 had additional malformations of the urinary tracts including urethral obstruction in 8 cases and double anlage in 5 patients

Children with uncomplicated reflux, i e without other malformations of the urinary tracts had as a rule a maximum of two episodes of urinary infection while children with complicated reflux had five or more episodes of urinary infection during the period of observation

The 38 children sustained a total of 1[illegible] urinary infections of which less than half were asymptomatic Pyrexia was the most common symptom in the remaining cases

Only 26 children were followed up for [a] minimum of 1 year In 12 children treated co[n]

servatively the reflux had ceased in four In 14 children a total of 18 ureters were implanted and the reflux was successfully relieved in six of these remained unchanged in 9 cases and in 3 cases no investigation has been performed since the last operation The reflux disappeared in 3 children only in the group submitted to operation in one case following implantation and in two following dilatation of the urethra for organic meatal stenosis

Discussion

Jørgen Vesterdal Dr Haahr s figures demonstrate how few of the children complain of dysuria and that pollakisuria was observed in only a few Although these symptoms are not observed in the majority of cases simply because the children are so small the investigation reveals nevertheless that investigation of the urine is essential in order to exclude infection as urinary infections are frequently symptomatic

I Hansted Is the treatment different for massive and for minimal reflux?

Haahr No differentiation was made in this material

C Højensgard (guest) Cystoscopy was undertaken only in a minority of the patients in the material presented This investigation is an important and necessary part of the urological investigation of children with recurrent urinary infections particularly in view of the indications for operation in cases of vesicoureteric reflux I do not consider that the investigation involves an undue risk of infection or trauma when compared with the serious nature of the underlying condition

Cystoscopy renders primarily important information concerning the appearance and function of the ureteric ostia. In severe cases of reflux large permanently gaping ostia are encountered There is definite difference between this form of malformation of the ostium and the ostium which is gaping on account of infective oedema of the mucosa. Frequently diverticulum of the bladder wall (sacculus) is observed at the site of the junction with the ureter suggesting insufficient mural support at the lower part of the ureter It is obviously important to note the location and number of the ureteric ostia Valuable information may be obtained by observation of the ureteric ostia during micturition which is an entirely different process from their behaviour during passive distension of the bladder employment of the abdominal musculature or pressure over the symphysis In many cases it is possible to obtain micturition around the cystoscope when the patient is waking up following suitable anaesthesia Finally cystoscopy renders information concerning other important conditions primarily signs of infection (particularly in cystitis cystica) and trabecular markings

At present uncertainty reigns as to the extent to which children with recurrent urinary infections should be submitted to operation and which technique should be employed In Kolding we have a group of patients in whom we consider operation is absolutely essential on the basis of the clinical picture (failure of medical treatment) radiographic findings (massive reflux with dilatation) and cystoscopy (ostia which are pathological in appearance and function) I shall not attempt to consider the operative methods here

N Hobolth As Højensgard has described we feel convinced that a differentiation must be made between slight and massive reflux In the latter filling of the entire ureter and the pelvis is observed in the micturition cystography and with practically no exceptions cystoscopy reveals either golf hole or stall door ostia In our material of 34 patients there were 14 cases of reflux 11 of which showed massive reflux in one or more ureters With the exception of a single patient who had very severe primary symptoms we waited for one or more recurrences to take place before undertaking operation All of the patients however were submitted to reflux inhibiting operation Numerous patients could be dis

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Only 26 children were followed up for a minimum of 1 year In 12 children treated con

BOOK REVIEWS

Ingrid Gamstorp *Pediatric neurology* Appleton Century-Crofts New York 1970 394 pp Illus 180s

Pediatric neurology is today a rapidly expanding field requiring increasingly specialized knowledge not least in diagnostic developmental medicine There is a great need for comprehensive and clinically oriented up-to-date informational material for post graduate education Ingrid Gamstorp a wellknown Swedish pediatric neurologist with a vast and broad based clinical experience has taken upon herself the large task of writing a book surveying the main topics that have to be known by everyone working with children suffering from neurological disorders or developmental delay Dr Gamstorp has chosen the very attractive and practically oriented approach of dividing her book into chapters according to the nature of the symptoms In addition to self-evident headings such as convulsions cerebral palsy mental retardation headache etc there are also interesting differential diagnostic titles such as "Generalized muscular hypotonia and flaccid weakness" "Abnormal muscle stiffness" "Localized weakness" "Ataxia" "Involuntary movements" etc Thus the book is really written for everyday practice in wards and out patient clinics In general the book is clearly written and gives adequate and up-to-date information It has well selected and modern references a good reference system between the various chapters and is extremely well illustrated This applies particularly to the chapter covering the neurologic and special examination and those treating neuromuscular weakness The section about mental retardation is less *well* balanced between common and very uncommon causes and lacks a didactic classification

In the introduction one seeks in vain for information as to what type of doctor this book has been directed To me it seems clear that this is the ideal textbook for the general pediatrician and the pediatrician under training who wants to learn more about the examination methods and the syndromes involved in pediatric neurology The same is also valid for the doctor with general neurological experience working with children In addition it is a good introductory source of information for those who really want to subspecialize in pediatric neurology However the compactness of the book 38_ printed pages tells us that the aim is not to be a substitute for complete and extensive specialized textbook of which Ford's work is the classic in the

It would be surprising if the reviewer agreed with the author on all points Dr Gamstorp has a tendency to be somewhat categoric in some of her statements even in recognizedly difficult and controversial fields of management where more than one approach or opinion might be correct. This applies for example to the treatment of subdural hygroma (p 224) where only the burr hole method is accepted subdural shunts not even being mentioned Her fears concerning diagnostic and therapeutic punctures seem rather exaggerated Further it is highly debatable whether "an examination of the cerebrospinal fluid *must* [reviewer's italics] be performed at least once in all children with findings suggesting cerebral palsy" the opposite view being most generally accepted among neuropediatricians It is furthermore not "only cerebellar forms of ataxia" (p 272) that are seen in cerebral palsy The CSF protein is not high "in all types of leucodystrophy" most of the sudanophilic cases having quite normal levels but an abnormal electrophoretic pattern The categorical and absolute rejection of phenobarbitone for use in older children with grand mal epilepsy is not correct in my opinion as a not insignificant number of such cases do very well for years on a single nightly dose of 50 mg of this drug Phenantoin or carbamazepin is not well tolerated nor ideal in all cases

Most of the figures given are accurate and based on good references One minor objection! The incidence of hydrocephalus (p 224) is 2–2 5 per 1 000 living newborns only in certain parts of the world owing to the large geographic variations in the incidence of spina bifida cystica Figures do exist for the incidence of peri and postnatally dependent simple hydrocephalus

The criticisms made above are however of minor importance In general this is a very useful and ambitious book which I think will help many pediatricians to a better understanding of a field which demands extensive and long clinical experience Dr Gamstorp has written a monograph with an exceptionally good clinical approach and with the ambition of trying to give her best advice for understanding and helping the suffering child and its parents in all situations She has indeed succeeded and this work should *find its way to the* book shelves of all pediatric and neurologic libraries

Bengt Hagberg

charged from treatment but the period of observation is not sufficiently long for any opinion to be expressed concerning the final result The operations were, however, successful in preventing further reflux

Meeting Dec 7 1970

Christmas meeting with talk by the author Palle Lauring on the conditions under which children have grown up during the centuries

Combined meeting with the Danish Society for Clinical Chemistry and Clinical Physiology Dec 12 1970 on Inborn errors of metabolism

N J Brandt *Laboratory problems in the diagnosis of inborn errors*

E Wamberg *Current and future screening methods for congenital metabolic diseases in newly born infants*

A Dupont *Screening for diseases involving oligophrenia in children in institutions*

J Clausen *Specific enzyme defects in glycolipid metabolism in the progressive encephalopathies (lysosome defects)*

Fl Guttler *Hyperphenylalaninaemia*

J Christensen & E Lykkegård Nielsen *Cystic Fibrosis Incidence and scereening*

Extraordinary meeting Dec 11, 1970

Sverre Halvorsen (Oslo) *Regulation of erythropoiesis in newly born infants by means of stimulators and inhibitors*

N J Brandt

W Th. Koos & M H Miller *Intracranial tumours of infants and children* Georg Thieme Verlag Stuttgart 1971 415 pp illus DM 130 —

The monograph presents a survey of current knowledge in the field of intracranial tumours in infants and children based on 700 brain tumour cases selected from the tumour collection of the Neurosurgical Clinic of University of Vienna The various conceptions of the authors have been influenced by personal experiences gathered in many years of clinical work at various neurosurgical centres both in the United States and in Europe

The book is divided into four main sections the first giving extensive statistical data and devoted to a detailed discussion of increased intracranial pressure and its various manifestations in the age groups concerned The histological classification of brain tumours, and tumours of neuroectodermal origin in particular is a difficult task and has been subjected to differences in opinion The authors have followed the classification of K. J Zulch based on studies of 6000 tumours The pathologist might find the photomicrographic material chosen as illustrating variations in the histologic appearance of tumours somewhat scarce and insufficient but the monograph is apparently not basically meant for the neuropathologist. The chapter dealing with increased intracranial pressure is well written and illustrated by excellent drawings schematically presenting possible variations in internal herniation at different sites

Parts II and III deal with general clinical diagnostic and therapeutic problems related to individual brain regions and with the special tumour types grouped by their topography It is quite obvious that special emphasis has been placed on diagnostic therapeutic methods modified and adapted to suit the particular conditions encountered in children of different ages, deviating from the techniques employed in adults The survey is brought up to date in every respect. The usefulness of such diagnostic aids as ultrasonography and radio-isotope scan is discussed and radiologic studies employing different contrast materials is beautifully illustrated with different types and locations of tumours The use of microneurosurgical techniques in dealing with parasellar lesions is advocated. In general the authors notion on surgical and postoperative treatment agrees entirely with current trends in major neurosurgical centres all over the world. The chapter on craniopharyngeomas to a large part reviews the experiences by Donald D Matson and co-workers published in 1969

The last part of the monograph is devoted to a description of chemotherapy of malignant tumours of the central nervous system and also includes a number of suitable references The authors do not state to what purpose their study was written but it seems to be suitable as supplementary reading in neurosurgery and should be included in the libraries of teaching institutions of pediatrics and neurosurgery

Lars Granholm

K. Decker & H Backmund *Pädiatrische Neuroradiologie* Georg Thieme Verlag Stuttgart 1970 193 pp illus DM 79 —

This book on neuroradiology in infancy and childhood is focused on the technical and diagnostic problems specific of paediatric disease of the central nervous system and it presupposes somewhat more than elementary knowledge in general neuroradiology The special procedures discussed include radio-isotope brain scanning as well as cerebral angiography encephalography ventriculography and myelography Attention is also given to the preparation of the patient and to general anaesthesia in connexion with these procedures

A separate chapter deals with the radiologic examination of a large series of human fetuses illustrating the prenatal development of the skull and vertebral column It includes observations made at ventriculography and angiography of several specimens providing useful information on the anatomy of the brain and its vasculature before birth This is followed by a description of the postnatal development and the range of normal variation of the central nervous system as reflected by the neuroradiological findings

The main part of the book is concerned with congenital and acquired neurologic disease including traumatic injury to the brain and spinal cord in early life The radiologic manifestation of such disease is elucidated by numerous references to paediatric, neurologic and radiologic literature and by the wide personal experience of the authors Numerous reproductions of radiographs help to throw light on this highly specialized and rapidly growing field of medicine

Georg Theander

G T Pack & A H Islami (eds) *Tumors of the liver* Recent results in cancer research Vol 26 Springer Verlag Berlin Heidelberg and New York 1970 304 pp illus DM 56 —

Like the previous volumes of the series Recent Results in Cancer Research this one is of a high standard presenting itself in an elegant outfit The subject tumors of the liver is covered from different points of view for example the epidemiology of primary carcinoma of the liver the pathologic anatomy of primary hepatic tumors hepatic carcinogenesis surgical anatomy of the liver radiation therapy of liver tumors surgical treatment of liver tumors transplantation of the liver and end results in the surgical treatment of liver tumors

It is unavoidable when many authors write the different chapters that these are of varying quality A survey on liver regeneration is extraordinarily well written by Bengmark Gothenburg The chapter of pathology of tumors of the liver in infancy and childhood is excellent too but one may wonder why the occurrence of precocious puberty associated with hepatoblastomas is not mentioned at all

The book is to be recommended to pathologists surgeons (including surgeons of pediatrics) and hepatologists as a thorough and well written work on *tumors of the liver*

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Gosta Rooth *Clinical acid-base and electrolyte balance* Studentlitteratur Lund 1970 80 pp Sw Cr 14 30

The present booklet by Gosta Rooth serves as a useful primer on several specific acid-base problems introducing quantitative views on respiratory compensation as well as the concepts of mixed acid-base disturbance and organic acidosis The subject matter is not treated systematically and preferably the book should be read as a continuation of Rooth Introduction to Acid-Base and Electrolyte Balance first published in 1966

The presentation is greatly simplified and largely maintained at a pre graduate level Hence the book should not be considered adequate for ward practice Having accepted the widely held misconception of acute deviations of blood base excess (BE) in response to primary changes in P_{CO} as artefacts Rooth bases all statements concerning changes in blood BE in terms of values computed for a fixed hemoglobin concentration of 50 g/L This convention is most unfortunate for a number of reasons Firstly it obscures the notion of BE as a measure of the blood concentration of (titratable) acid and introduces inconsistencies between stated chemical variables of the sample medium Secondly it invites violation of sound chemical data by application of *interpretative* corrections (in the present case based on false assumptions regarding the stability of the slope of the so called *in vivo* CO_2 absorption line) Thirdly the distributional effect on the blood acid-base status is physiologically relevant and should be registered And finally the use of a BE_{nb-s} in the description of primary non respiratory disturbances is conceptually misleading

Still this book offers the uninitiated several valuable short cuts to the understanding of clinical acid-base problems According to the author it is intended for doctors who cannot spend more than 3-5 hours on a new book To those it may be recommended

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Jerome L Schulman describes interesting experimental work in mice aiming at the evaluation of live influenza virus vaccine The importance of monitoring the antigenic drift of neuraminidase proteins and of evaluating vaccines in terms of their capacity to elicit antibodies to neuraminidase as well as to hemagglutinin are stressed The author emphasizes that research should continue for an effective live influenza virus vaccine

Stick and Yohn present an informative survey of the evidence that mammalian viruses are mutagenic agents and do induce chromosomal and mitotic irregularities The authors point out the need for greater attention to the question of whether vaccination with live attenuated viruses might result in any significant increase of genetically abnormal cells or not

The majority of the contributions deal with basic virology at the level of viral nuclear acids The rapid progress of knowledge including methodological innovations and the new concepts of molecular virology make the review of such topics necessary The book is of particular interest to those interested in these special segments of virology

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General Index

Authors Index

ANNOUNCEMENT

VIII MEDITERRANEAN AND MIDDLE EASTERN PAEDIATRIC CONGRESS

This Congress sponsored by the Union of the Mediterranean and Middle Eastern Paediatric Congress and the Spanish Paediatric Society will be held in Barcelona (Spain) from 12th till 15th of March 1972 at the Children s Hospital of the Seguridad Social President Dr A Ballabriga

Three main topics will be discussed

Newborns

Nutritional Problems in Childhood

Cancer and Leukaemia in Childhood

Several round tables and symposia upon other topics are also contemplated

Official languages Spanish English and French Simultaneous translation will be provided

For advance program detailing the planning and organizing of the Congress and providing all symposium and workshop titles advance registration and hotel reservations forms write to Secretariat VIII Mediterranean and Middle Eastern Paediatric Congress Dr A Gallart Clinica Infantil de la Seguridad Social Paseo Valle de Hebron s/n Barcelona Spain

General Index

Authors Index

Subject Index

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